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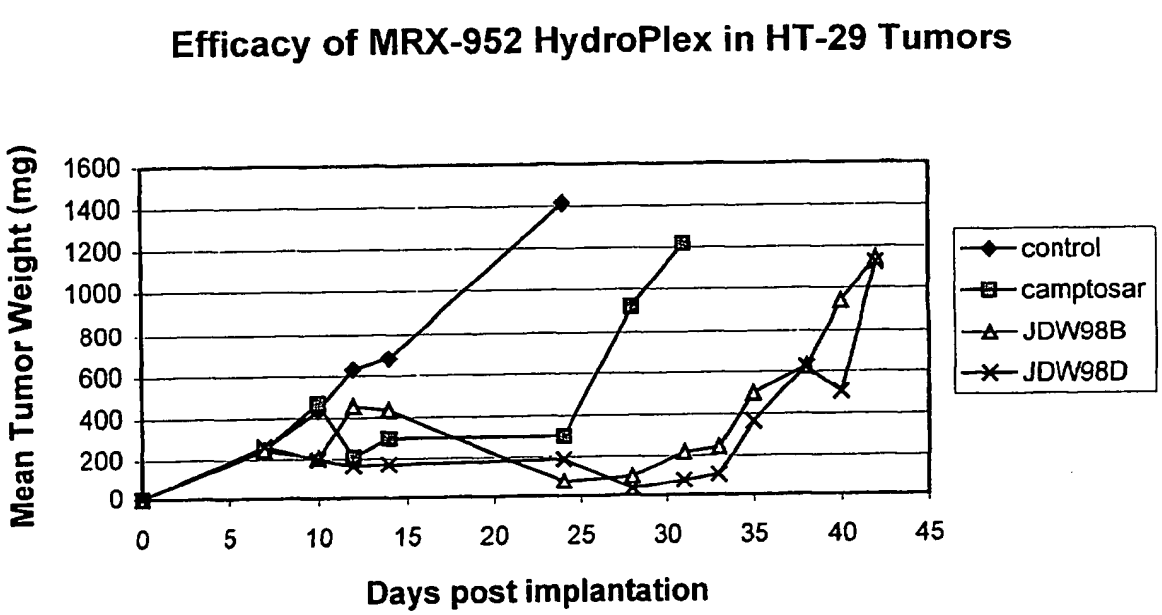
(54) Title: STABILIZED NANOPARTICLE FORMULATIONS OF CAMPTOTHECA DERIVATIVES



(57) Abstract: Pharmaceutical formulations are provided that increase the systemic bioavailability of camptotheca derivatives; preferably, the camptothecin derivative is 7-ethyl-10-hydroxyl camptothecin, SN-38. The drug is complexed with a stabilizing agent, but is not covalently bound thereto. Anionic or neutral lipids and/or polymers are used as the stabilizing agent, and secondary stabilizing agents and/or other excipients may be incorporated into the formulation as well. Therapeutic methods are also provided, wherein a formulation of the invention is administered to a patient to treat a condition, disorder, or disease that is responsive to camptothecin derivatives. Generally, administration is oral or parenteral.

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STABILIZED NANOPARTICLE FORMULATIONS OF CAMPTOTHECA DERIVATIVES

TECHNICAL FIELD

[0001] The present invention relates generally to pharmaceutical formulations, and more particularly to pharmaceutical formulations containing nanoparticles that are preferably amorphous or noncrystalline, stabilized by polymers and/or lipids for the delivery of camptothecin analogs, preferably SN-38. The invention has utility in the fields of pharmaceutical formulation, drug delivery, and medicine.

BACKGROUND ART

[0002] Camptothecin is an antineoplastic drug that acts an inhibitor of DNA topoisomerase I. Several natural and synthetic analogs of camptothecin have been identified and tested for antineoplastic efficacy. Camptothecin derivatives, as a class, are insoluble in aqueous solvents, unless modified with polar or ionic groups, as is done with CPT-11. Thus, one approach to the pharmaceutical formulation of camptothecin derivatives has been to covalently modify them with polar or charged moieties to increase their water solubility. Drawbacks to this approach include reductions in therapeutic potency and the expense involved in synthesizing analogs.

[0003] The formulation and administration of water-insoluble or sparingly water-soluble drugs, such as camptothecin and camptothecin analogs, are problematic in general because of the difficulty of achieving sufficient systemic bioavailability. Low aqueous solubility results not only in decreased bioavailability, but also in formulations that are insufficiently stable over extended storage periods. For the most part, research has focused on entrapment of the drug in vesicles or liposomes, and on the incorporation of surfactants into camptothecin formulations.

[0004] Representative liposomal drug delivery systems are described in U.S. Patent No. 5,395,619, U.S. Patent No. 5,340,588, and U.S. Patent No. 5,154,930. Liposomes, as is well known in the art, are vesicles comprised of concentrically ordered lipid bilayers that encapsulate an aqueous phase. Liposomes form when phospholipids (amphipathic compounds having a polar (hydrophilic) head group covalently bound to a long-chain aliphatic (hydrophobic) tail) are exposed to water. That is, in an aqueous medium, phospholipids aggregate to form a structure in which the long-chain aliphatic tails are

sequestered within the interior of a shell formed by the polar head groups. Unfortunately, use of liposomes for delivering many drugs has proven unsatisfactory, in part because liposome compositions are, as a general rule, rapidly cleared from the bloodstream. Finally, even if satisfactory liposomal formulations could be prepared, it might still be necessary to use some sort of physical release mechanism so that the vesicle releases the camptothecin analog in the body before the liver and spleen take up the agent.

[0005] Specifically regarding liposomal delivery of camptothecin, Burke (U.S. Patent No. 5,552,156) describes liposomes that have molar ratios of lipid to drug in the range of 1,000:1 to 100,000:1, or that have lipid concentrations of approximately 0.29 M. This range of ratios does not optimize the amount of antineoplastic agent for therapeutic administration, and the concentration makes stable vesicle formation problematic. Secondarily, liposome clearance would not allow for extended periods of bioavailability, without engineering the liposomes to avoid the reticuloendothelial system. While the use of PEGylated liposomes partially resolves this dilemma, the amounts of lipids needed for administration may give rise to acute toxicity.

[0006] Micelles also have been used for drug delivery, as exemplified by the disclosure in U.S. Patent No. 5,736,156 regarding camptothecin. Micelles are defined as spherical receptacles comprised of a single monolayer defining a closed compartment. Generally, amphipathic molecules such as surfactants and fatty acids spontaneously form micellar structures in polar solvents. In contrast to liposome bilayers, micelles are "sided" in that they project a hydrophilic, polar outer surface and a hydrophobic interior. Since they are monolayers, they are extremely limited in size, seldom exceeding 30 nanometers in diameter. This limited size reduces their effective encapsulation potential as drug carriers.

[0007] Among other notable drug delivery formulations, nanocrystals of drugs or carrier-stabilized drugs have been described in the art (for example, in U.S. Patent No. 5,399,363 to Liversidge et al.). Liversidge et al. describe the production of nanoparticles of hydrophobic drugs, including natural camptothecin, using surfactants and grinding. They mention a number of surfactants, including poloxamers, and list lecithin as a stabilizing material, but provide no disclosure of types of lipids or specific formulations containing polymers and lipids. They also never mention Camptotheca alkaloids other than camptothecin. Also, while the formulations disclosed by Liversidge et al. provide a way for maximizing drug delivery capacity, their crystalline nature is problematical because of the well-known phenomenon of

crystal growth over time. To overcome crystal growth, nanoparticulate crystals are sometimes coated with crystal growth-inhibiting agents such as nonionic surfactants. In these instances, care must be exerted to insure biocompatibility and nontoxicity of the surfactant or other coating agent.

[0008] Another way to improve drug delivery is to formulate medications into nanoparticles. By so doing, for example, hydrophobic or toxic drugs can be more safely delivered. The nanoparticles used for such purposes should be as small as possible, preferably less than 100 nanometers in diameter. Tumors for example, contain leaky blood vessels from which nanoparticles that comprise an antineoplastic medication may extravasate, i.e., the nanoparticles may leak out of the blood vessels into the interstitial space of the tumor tissue.

[0009] Collectively, there remains a need in the art for a pharmaceutical formulation that is suitable for administration of a water-insoluble or sparingly water-soluble drugs such as camptothecin or its analogs, wherein (1) the formulation is optimized such that the amount of drug administered is maximized while undesirable side effects are minimized, (2) the rate of drug release can be precisely controlled, (3) no micelles, liposomes, or other vesicles are required, (4) premedication is unnecessary, and (5) the formulation displays excellent stability over extended storage periods.

[0010] Among the synthetic analogs and derivatives of camptothecin, 7-ethyl-10-hydroxycamptothecin, designated SN-38, has generated considerable recent interest. It is a metabolite of another synthetic analog, irinotecan, and has demonstrated antineoplastic efficacy somewhat greater that of camptothecin and some of the other analogs. Further, SN-38 has a longer serum half-life than that of natural camptothecin. SN-38 is hydrophobic, and up until now has been difficult to formulate as a drug for therapeutic use in humans. A pro-drug of SN-38, called irinotecan, has been developed for use in humans and is approved for treatment of colon cancer. Unfortunately, irinotecan is associated with adverse side effects, including severe diarrhea. Additionally, irinotecan, as a pro-drug, irinotecan must be converted to the active SN-38 molecule by carboxylesterases in the body. Not all tumors contain sufficient carboxylesterases to form the potent SN-38 drug. It thus appears that SN-38 could be an effective anticancer agent, if it was formulated properly for administration to patients. An improved formulation of SN-38 might also be better tolerated with fewer side

effects; in particular, it might be better tolerated in elderly and sick patients, with resulting improvements in efficacy and treatment response.

[0011] The instant invention addresses those needs by providing unique formulations of camptothecin analogs that have improved properties useful in drug delivery.

DISCLOSURE OF THE INVENTION

[0012] One aspect of the invention relates to addressing the above-mentioned needs in the art by providing a pharmaceutical formulation effective to deliver a camptothecin analog.

[0013] Another aspect of the invention pertains to a therapeutic method wherein the aforementioned formulation is administered to a patient to treat a condition, disease, or disorder for which the drug is indicated.

[0014] In one aspect of the invention, then, a pharmaceutical formulation is provided that comprises a camptothecin analog, a stabilizing agent that stabilizes the camptothecin analog but does not covalently bind thereto, an optional targeting ligand, an optional secondary stabilizing agent, and an optional excipient. A variety of stabilizing agents may be employed, although polymers, such as poloxamine, poloxamer, polyethylene glycol and poly(ethylene oxide-co-propylene oxide), branched block copolymers, and/or neutral and/or anionic lipids, such as phospholipids and lecthins, are preferred. The most preferred formulations comprise both polymeric stabilizing agents and lipidic and/or PEG-lipid stabilizing agents.

[0015] The formulation may be in lyophilized form, which is advantageous for storage stability. The formulation may also be in the form of an aqueous suspension and may further comprise an aqueous vehicle. The aqueous vehicle may be, for example, water, isotonic saline solution, isotonic dextrose or phosphate buffer, and may be instilled with an acoustically active gas to facilitate ultrasound imaging and ultrasonic cavitation for local drug release with ultrasound.

[0016] In another aspect of the invention, a method is provided for making the aforementioned formulation, comprising the steps of (1) admixing, in a solvent, a camptothecin analog and a stabilizing agent that stabilizes the camptothecin analog but does not covalently bond thereto; (2) removing the solvent in a manner effective to provide a dry formulation of the camptothecin analog; and (3) rehydrating the dry formulation to form the nanoparticulate formulation. In this method, the solvent may be removed by lyophilization, spray drying, super critical fluid processing or rotary evaporation.

[0017] In another aspect of the invention, a method is provided for delivering a drug to a mammalian individual to achieve a desired therapeutic effect, wherein the method involves administering to the individual a therapeutically effective amount of a formulation of the invention, e.g., intravenously, orally, parenterally, intraperitoneally, subcutaneously or via injection into a body cavity such as a joint, or via inhalation for delivery to the lungs.

[0018] In a related aspect of the invention, a method is provided for treating an individual suffering from cancer, comprising parenterally administering to the patient a spatially stabilized matrix formulation of: (a) drug-containing particles comprised of (i) a stabilizing agent, (ii) a camptothecin analog that complexes with but does not covalently bind to the stabilizing agent, optionally (iii) a targeting ligand, and optionally (iv) an excipient selected from the group consisting of saccharides, liquid polyethylene glycols, propylene glycol, glycerol, ethyl alcohol, and combinations thereof, in (b) an aqueous vehicle.

[0019] The present invention is based on the formation of a noncovalent complex of a camptothecin analog with a stabilizing agent. The drug/polymer complex is a spatially stabilized matrix. A unique feature of this complex is that nanoparticles are formed only in the presence of drug and stabilizing material. This drug/polymer complex allows for the formation of nanoparticles that may be suspended in an aqueous solution, without requiring chemical modification of the camptothecin analog. This nanoparticle solubilization technology enables the preparation of camptothecin analog formulations with decreased toxicity and improved efficacy. The well-documented problems related to stability, carrier toxicity, and large injection volume of currently available formulations of camptothecin analogs are eliminated with this novel technology.

[0020] The noncovalent complex of a camptothecin analog with a stabilizing agent produces a unique class of nanoparticles ranging from about 1 nanometer to about 2000 nanometers, preferably from about 200 nm to about 500 μm, that can be further treated with a second stabilizing agent to form nanoparticles having diameters ranging from about 1 nm to about 300 nm, preferably from about 20 nm to about 100 nm. The resulting nanoparticles are biocompatible and highly useful for drug delivery. The drug delivery is preferably via intravenous (IV) injection, but the technology has applications for oral, subcutaneous (e.g., sustained release), and pulmonary delivery. For IV delivery, the nanoparticles are useful in that they can, for example, decrease the toxicity of therapeutic agents. Compared to existing methods, larger doses of the camptothecin analogs can therefore be administered

intravenously, allowing for higher blood levels of the therapeutic agent, which can yield greater efficacy. For oral applications, the nanoparticles improve the dispersal of camptothecin analogs and increase uptake from the gastrointestinal tract. For sustained release applications, the nanoparticles can be formulated into gels, powders, or suspensions. For pulmonary applications, the nanoparticles' small effective hydrodynamic radii improve the delivery of therapeutic agents into the distal airways, such as the alveoli, thereby allowing systemic delivery of camptothecin analogs via the pulmonary route. In this regard, pulmonary delivery is also useful for local treatment of lung cancer, particularly alveolar cell carcinoma.

[0021] Additional aspects, advantages, and features of the invention will be set forth in part in the description that follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 is a graph presenting relative tumor size as a function of the time following treatment, for treatment with two formulations of SN-38, irinotecan, and for a non-treated control.

[0023] FIG. 2 is a graph presenting relative tumor size as a function of the time following treatment for various lipid stabilized SN-38 formulations of the invention.

MODES FOR CARRYING OUT THE INVENTION

A. DEFINITIONS AND ABBREVIATIONS

[0024] It is to be understood that unless otherwise indicated, this invention is not limited to specific camptothecin analogs, hydrophilic polymers, copolymers, phospholipids, excipients, methods of manufacture, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0025] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a camptothecin analog" or "a drug" in a formulation means that more than one camptothecin analog can be present, reference to "a stabilizing agent" includes combinations of stabilizing agents, reference to "a phospholipid" includes mixtures of phospholipids, and the like.

[0026] In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

[0027] By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected camptothecin analog without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

[0028] "Pharmaceutically or therapeutically effective dose or amount" refers to a dosage level sufficient to induce a desired biological result. That result can be alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system.

[0029] The term "treat" as in "to treat a disease" is intended to include any means of treating a disease in a mammal, including (1) preventing the disease, i.e., avoiding any clinical symptoms of the disease, (2) inhibiting the disease, that is, arresting the development or progression of clinical symptoms, and/or (3) relieving the disease, i.e., causing regression of clinical symptoms.

[0030] The terms "disease," "disorder," and "condition" are used interchangeably herein to refer to a physiological state that may be treated using the formulations of the invention.

[0031] The number given as the "molecular weight" of a compound, as in the molecular weight of a hydrophilic polymer such as polyethylene glycol, refers to weight average molecular weight M_w.

[0032] "Lipid" refers to a synthetic or naturally-occurring compound which is generally amphipathic and biocompatible. The lipids typically comprise a hydrophilic component and a hydrophobic component. Exemplary lipids include, for example, fatty acids, neutral fats, phospholipids, phosphatides, glycolipids, surface-active agents, aliphatic alcohols, and steroids. Specifically, the choice of the term is used to distinguish it from the more stringently defined terms "liposome" and "micelle," wherein a liposome implies a vesicular structure with a defined interior aqueous compartment. The arrangement of molecules in a liposome gives rise to a vesicle of at least one lamellar bilayer. Drugs may be sequestered within the interior of liposomes, embedded within the lipid matrix, or affixed to the outside surface of the liposome. In a micelle, there is an arrangement of polar amphipathic molecules, wherein the hydrophilic portion (heads) of the structure defines the exterior surface and the hydrophobic portion (tails) resides interiorly, away from the medium. A

micelle is not, by definition, a bilayer, and thus its size and effective carrying capacity is limited according to properties defined by the critical micelle concentration for a given compound. In contrast to liposomes and micelles, lipidic structures are non-liposomal, non-micellar associations of lipid and drug.

[0033] The term "lecithin" refers the class of phospholipids called phosphatidylcholines, and generally refers to natural phosphatidylcholines such as dioleylphosphatidylcholine. Such naturally occurring phospholipids are composed of phosphate, choline, glycerol (as the ester), and two fatty acids, and are exclusively modified with phosphatidylcholine at the 3-position of the glycerol. The fatty acyl moieties attached at the 1 and 2 hydroxyl positions of glycerol may be saturated, unsaturated, or a combination of both. Lecithin does not comprise anionic phospholipids such as phosphatidylglycerol, or chemically modified, synthetic phospholipids.

[0034] "Polymer" refers to molecules formed from the chemical union of two or more repeating units. Accordingly, included within the term "polymer" may be, for example, dimers, trimers and oligomers. The polymer may be synthetic, naturally-occurring or semisynthetic. In one embodiment, the term "polymer" refers to molecules which comprise 10 or more repeating units. In other embodiments, the polymers which may be incorporated in the compositions described herein contain no denatured naturally occurring proteins that are crosslinked by disulfide linkages.

[0035] "Covalent association" refers to an intermolecular association or bond which involves the sharing of electrons in the bonding orbitals of two atoms.

[0036] "Non-covalent association" refers to intermolecular interaction among two or more separate molecules which does not involve a covalent bond. Intermolecular interaction is dependent upon a variety of factors, including, for example, the polarity of the involved molecules, the charge (positive or negative), if any, of the involved molecules, and the like. Non-covalent associations are preferably selected from the group consisting of ionic interaction, dipole-dipole interaction and van der Waal's forces and combinations thereof.

[0037] "Targeting ligand" refers to any material or substance which may promote targeting of tissues and/or receptors *in vivo* with the compositions described herein. The targeting ligand may be synthetic, semi-synthetic, or naturally-occurring. Materials or substances which may serve as targeting ligands include, for example, proteins, including antibodies, glycoproteins and lectins, peptides, polypeptides, saccharides, including mono-

and polysaccharides, vitamins, steroids, steroid analogs, hormones, cofactors, bioactive agents, prostacyclin and prostaglandin analogs, and genetic material, including nucleosides, nucleotides and polynucleotides.

[0038] "Peptide" or "polypeptide" refers to nitrogenous polymeric compounds which may contain from about 2 to about 100 amino acid residues. In certain embodiments, the peptides which may be incorporated in the compositions described herein contain no denatured naturally occurring proteins that are crosslinked by disulfide linkages.

[0039] "Protein" refers to a nitrogenous polymer compound which may contain more than about 100 amino acid residues. In certain embodiments, the proteins which may be incorporated in the compositions described herein contain no denatured naturally occurring proteins that are crosslinked by disulfide linkages.

[0040] "Nanoparticles" are defined strictly according to size in that they have diameters less than one micrometer. The term may embrace amorphous, structured, or partially crystalline forms. "Nanocrystals" by contrast are defined as structures with sizes less than one micrometer, but that have at least 99% crystalline structure, regardless of whether the molecular composition of said crystal is purely one component, e.g., drug, or drug in close association with another component.

[0041] The term "stabilizer" refers to materials such as lipids, polymers, polymer-lipid conjugates, and other coating agents, surfactants, or compounds that alter the physical and chemical properties affecting aqueous solubility of a drug when placed in a noncovalent admixture with the drug or drugs.

[0042] The "solubility" of a compound refers to its solubility in the indicated liquid determined under standard conditions, e.g., at room temperature (typically about 25°C), atmospheric pressure, and neutral pH.

[0043] In referring to chemical compounds herein, the following definitions apply:

[0044] The term "alkyl" refers to a branched or unbranched saturated hydrocarbon group of 1 to 24, typically 10 to 20, carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like, as well as cycloalkyl groups such as cyclopentyl, cyclohexyl, and the like.

[0045] The term "aryl" refers to an aromatic species containing 1 to 3 aromatic rings, either fused or linked, and either unsubstituted or substituted with one or more substituents. Preferred aryl substituents contain one aromatic ring or two fused aromatic rings.

[0046] The term "acyl" refers to a group having the structure R(CO)- wherein R is alkyl or aryl as defined above.

[0047] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

B. FORMULATIONS

[0048] The pharmaceutical formulations of the invention are advantageously used to deliver camptothecin analogs by increasing the solubility of the drug in water. The instant invention described herein discloses compositions and methods for making and using preferably noncrystalline, lipidic, and/or polymeric nanoparticles for delivery of camptothecin analogs, preferably SN-38. In some cases, the particles are non-birefringent, indicating amorphous nanoparticles. As the lipid content is decreased, the particles may be birefringent. Because of the way that the particles are made, however, it is believed that when birefringent structures are present, these structures contain substantially more than 0.1% of a second material (e.g., polymer or lipid) interspersed within the drug matrix.

[0049] Generally, the noncovalent complex of the camptothecin analog and stabilizing agent produces nanoparticles ranging from about 1-2000 nanometers, preferably from about 200 nm to about 500 μm. In one embodiment, the formulation represents a unique class of nanoparticles ranging from about 1 nm to about 10-1000 nm, preferably from about 100 nm to about 900 nm, that can be stabilized with a stabilizing agent, preferably lipidic and/or polymeric, to form nanoparticles having diameters ranging from about 200 nm to about 800 nm. Another preferred range is about 50-800 nm. The resulting nanoparticles are biocompatible and highly useful for drug delivery. The drug delivery is preferably via IV injection, but the technology has applications for oral, subcutaneous (e.g., sustained release), intracisternal, intranasal, and pulmonary delivery. For IV delivery, the nanoparticles increase the stability and bioavailability of the camptothecin analogs. For example, small particles, by virtue of their larger accessible surface-to-volume ratio, tend to release drug quite rapidly, while larger particles, will provide for far more gradual, sustained release of drug. For pulmonary administration, particle size is optimally within the range of about 500 to 5,000 nm. For intramuscular and subcutaneous injection, particle size should be in the range of about 1 nm to 10,000 nm. For intravenous administration, particle size is optionally in the

range of about 10 nm to 1,000 nm, preferably about 200 to 800 nm. For interstitial administration and fracture or wound packing, and for embolization, particle size can be up to 10,000 nm.

[0050] In the preferred embodiment of the invention, the nanoparticles of the invention are further characterized in that crystal content is substantially under 99%, and generally well under 50%, for most formulations described herein. By this it is meant, that while some lipid or other stabilized formulations show birefringence characteristic of the presence of crystals, the mole fraction of drug in the formulation and the strong noncovalent interaction of lipidic molecules and/or amphipathic polymers with the drug, limit the amount of drug-drug molecular interactions, thus limiting the relative proportion of the drug in crystalline form and the size of the crystallites. These phenomena provide obvious advantages over other formulations described in the art in terms of size stability.

[0051] In still another embodiment of the invention, the stabilizing agent may be a combination of a lipidic and/or PEG-lipid conjugated material and a polymeric material such as poloxamine, poloxamer, branched block copolymer or polyethylene glycol in a variety of ratios.

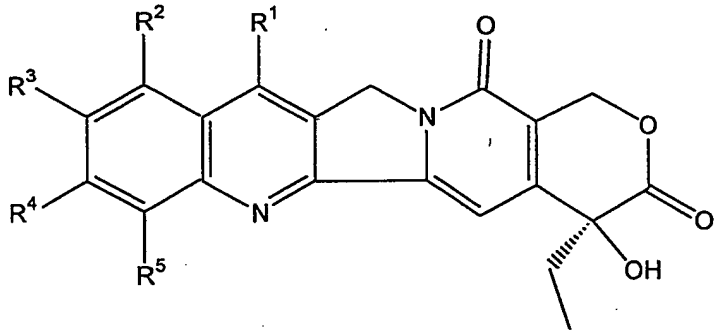
I. THE CAMPTOTHECIN ANALOG

[0052] The drug in the formulation, as noted above, is any camptothecin analog (the "active agent") whose systemic bioavailability can be enhanced by increasing the dispersability of the agent in water. It will be appreciated that the invention is particularly useful for delivering camptothecin analogs for which chronic administration may be required, as the present formulations provide for sustained release. The invention thus has the advantage of substantially improving patient compliance, as the potential for missed or mistimed doses is greatly reduced. However, any agent that is typically incorporated into a capsule, tablet, troche, liquid, suspension, or emulsion, wherein administration is on a regular schedule (i.e., daily, more than once daily, every other day, or any other regular interval) can be advantageously delivered using the formulations of the invention.

[0053] Camptothecin analogs are topoisomerase I inhibitors, such as Camptotheca alkaloids including, but not limited to, homocamptothecin, diflomotecan, exatecan, SN-38, topotecan, irinotecan, and carzelesin, and pharmaceutically acceptable salts of any of the above. Especially preferred are derivatives at the 9 ring position, including 9-nitro-

camptothecin and 9-amino-camptothecin; modified 10-position compounds, including 10-hydroxycamptothecin and aminated, aminoalkyl, alkylated, and alkoxylated derivatives of the same; modified 11-position derivatives, including 11-hydroxycamptothecin and aminated, aminoalkyl, alkylated, and alkoxylated derivatives of the same; modified 12-position derivatives, including 12-hydroxycamptothecin and aminated, aminoalkyl, alkylated, and alkoxylated derivatives of the same; 7-position derivatives, including amino, nitro, alkyl, alkylamino, and alkoxy derivatives of the same; and 20 (S) derivatives, including alkylesters and amides of the 20-(OH) group. Preferred compounds within this group include 7-alkylcamptothecin and especially 7-ethyl camptothecin. Other derivatives include permutations of combinations of the above compounds wherein any or all of the 7, 9, 10, 11, and 20(OH) positions may be modified. Among these, a preferred compound and derivative is 7-ethyl-10-hydroxycamptothecin, designated SN-38, a metabolite of irinotecan. One of skill in the art will readily appreciate that any or all functional groups on camptothecin or its analogs are amenable to derivatization into prodrugs, including but not limited to the 9, 10, 11, and 20 ring substituents, with derivatization at the 20 position preferred.

[0054] Camptothecin analogs suitable for use in the present formulation may be represented by the structure of formula (I)



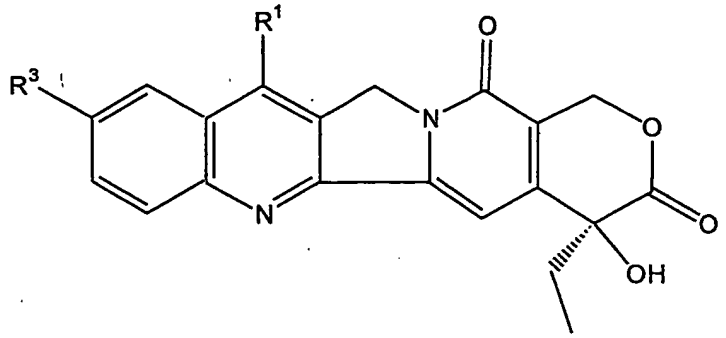
(I)

wherein R^1, R^2, R^3, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6} alkylamido, amino, nitro, and cyano; or R^1 and R^2 and/or R^3 and R^4 may taken together to form a substituted or unsubstituted five- or six-membered cyclic group containing up to two heteroatoms selected from the group consisting of O, S, and N.

[0055] Preferably, R^1, R^2, R^3, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6}

alkylamido, amino, nitro, and cyano. In further preferred embodiments, R^1 is C_{1-6} alkyl; most preferably, R^1 is methyl. Preferably, R^3 is hydroxyl.

[0056] In other preferred embodiments, R^2, R^4, and R^5 are H, such that the camptothecin analog has the structure of formula (II)



(II)

wherein R^1 is C_{1-6} alkyl and R^3 is hydroxyl, sulfhydryl, or amino. Most preferably, R^1 is methyl, R^3 is hydroxyl, and the camptothecin analog is 7-ethyl-10-hydroxyl camptothecin, SN-38.

[0057] The amount of camptothecin analog in the formulation should be such that the weight ratio of analog to all other components of the formulation is in the range of about 1:1 to 1:200, preferably in the range of about 1:10 to 1:100, more preferably in the range of about 1:20 to 1:75, and optimally about 1:30 to 1:50.

II. THE STABILIZING AGENT

[0058] The stabilizing agents of the present invention are polymers, lipids, polymer-lipid conjugates, or combinations thereof, that are capable of forming noncovalent complexes with the camptothecin analogs.

A. POLYMERS

[0059] The polymers can be linear, or branched structures, including block copolymers and branched block copolymers. It should be understood that the term "branched", when applied to polymers, also includes any dendritic, star, or star-like polymer. Useful polymers for stabilizing the nanoparticles include linear or branched polyethylene glycol (PEG), and copolymers of PEG with polypropylene oxide, such as the PLURONICS® (BASF Corporation, Mount Olive, NY). Linear block polymers are poloxamer, a block copolymer of propylene oxide flanked on each end by ethylene oxide; and poloxamine, a polyalkoxylated symmetrical block polymer of ethylene diamine conforming to the general type $[(PEG)_X$-

$(PPG)_Y]_2$-NCH_2CH_2N-$[(PPG)_Y$-$(PEG)_X]_2$. Preferred species of poloxamer are the PLURONICS®, with PLURONIC® F68 being highly preferred. Suitable poloxamines include the TETRONICS® with TETRONIC® 908 is a preferred species with a molecular weight of 25,000 daltons. Other derivatives with shorter PEG and PPG copolymeric chains having molecular weights between 1650 daltons to 25 kilodaltons are also suitable. Branched block copolymers are especially useful as stabilizing agents, particularly those with a molecular weight of 8000 to 15000 daltons containing both hydrophilic and hydrophobic blocks. These branched block copolymers may be comprised of either a hydrophobic core and hydrophilic distal arms, or a hydrophilic core and hydrophobic distal arms, and are described in greater detail below.

[0060] In one embodiment, the compositions of the present invention comprise hydrophilic and/or hydrophobic polymers, with hydrophilic polymers being preferred. The term "hydrophilic," as used herein, refers to a composition, substance or material, for example, a polymer, which may generally readily associate with water. Thus, although the hydrophilic polymers that may be employed in the present invention may have domains of varying type, for example, domains which are more hydrophilic and domains which are more hydrophobic, the overall nature of the hydrophilic polymers is preferably hydrophilic, it being understood, of course, that this hydrophilicity may vary across a continuum from relatively more hydrophilic to relatively less hydrophilic. The term "hydrophobic," as used herein, refers to a composition, substance or material, for example, a polymer, which generally does not readily associate with water. Thus, although the hydrophobic polymers that may be employed in the present invention may have domains of varying type, for example, domains which are more hydrophobic and domains which are more hydrophilic, the overall nature of the hydrophobic polymers is preferably hydrophobic, it being understood, of course, that this hydrophobicity may vary across a continuum from relatively more hydrophobic to relatively less hydrophobic.

[0061] In some embodiments, the polymer stabilizing agent may be in the form of a matrix or three-dimensional structure which may be spatially stabilized. The term "matrix," as used herein, refers to a three dimensional structure which may comprise, for example, a single molecule of a polymer, such as PEG associated with one or more molecules of the active drug, or a complex comprising a plurality of polymer molecules in association with the active drug. The morphology of the matrix may be, for example, particulate, where the

particles are preferably in the form of nanoparticulate structures. The term "spatially stabilized," as used herein, means that the relative orientation of the active agent, when present in the matrices, may be fixed or substantially fixed in three-dimensional space, without directional specification. Thus, compositions described herein may facilitate physical entrapment and, preferably, immobilization or substantial immobilization, of the camptothecin analog. Generally, although not necessarily, the spatially stabilized matrix may be sterically constrained. In one embodiment, the matrices are hydrophilic, i.e., the overall nature of the matrices is hydrophilic.

[0062] Stability may be evaluated, for example, by placing the pharmaceutical composition in water, and monitoring for dissolution and/or release of the active drug. Preferably, the pharmaceutical compositions may be spatially stable for at least about 5 minutes, more preferably at least about 30 minutes, even more preferably for more than an hour. In certain embodiments, the pharmaceutical compositions may be spatially stable in solution for days, weeks, and even months.

[0063] In certain embodiments, the present matrices may comprise a network of particulate structures. The size and shape of the particulate structures may vary depending, for example, on the particular polymer employed, the desired rate of release of the active drug, and the like. For example, the particulate structures may be spherical in shape, or they may take on a variety of regular or irregular shapes. With regard to the size of the particles, in one embodiment, the diameter of the particles may range from about 1 nanometer (nm) to about 1000 nm, and all combinations and subcombinations of ranges and specific particle sizes therein.

[0064] A wide variety of polymers may be employed in the present compositions and formulations. Generally speaking, the polymer is one which has the desired hydrophilicity and/or hydrophobicity, and which may form matrices, as well as covalent attachments with targeting ligands, as described in detail herein. The polymer may be crosslinked or non-crosslinked, with substantially non-crosslinked polymers being preferred. The terms "crosslink," "crosslinked," and "crosslinking," as used herein, generally refer to the linking of two or more compounds or materials, for example, polymers, by one or more bridges. The bridges, which may be composed of one or more elements, groups or compounds, generally serve to join an atom from a first compound or material molecule to an atom of a second compound or material molecule. The crosslink bridges may involve covalent and/or non-

covalent associations. Any of a variety of elements, groups and/or compounds may form the bridges in the crosslinks, and the compounds or materials may be crosslinked naturally or through synthetic means. For example, crosslinking may occur in nature in materials formulated from peptide chains which are joined by disulfide bonds of cystine residues, as in keratins, insulin, and other proteins. Alternatively, crosslinking may be effected by suitable chemical modification, such as, for example, by combining a compound or material, such as a polymer, and a chemical substance that may serve as a crosslinking agent, which are caused to react, for example, by exposure to heat, high-energy radiation, ultrasonic radiation, and the like. Examples include, for example, crosslinking with sulfur which may be present, for example, as sulfhydryl groups in cysteine residues, to provide disulfide linkages, crosslinking with organic peroxides, crosslinking of unsaturated materials by means of high-energy radiation, crosslinking with dimethylol carbamate, and the like. The term "substantially," as used in reference to crosslinking, means that greater than about 50% of the involved compounds or materials contain crosslinking bridges. In certain embodiments, greater than about 60% of the compounds or materials contain crosslinking bridges, with greater than about 70% being a preferred embodiment. Even more preferably, greater than about 80% of the compounds or materials contain crosslinking bridges, with greater than about 90% being still more preferred. In certain embodiments, greater than about 95% of the compounds or materials contain crosslinking bridges. If desired, the substantially crosslinked compounds or materials may be completely crosslinked (i.e., about 100% of the compounds or materials contain crosslinking bridges). In other embodiments, the compounds or materials may be substantially (including completely) non-crosslinked. The term "substantially," as used in reference to non-crosslinked compounds or materials, means that greater than about 50% of the compounds or materials are devoid of crosslinking bridges. In a preferred embodiment, greater than about 60% of the compounds or materials are devoid of crosslinking bridges, with greater than about 70% being more preferred. Even more preferably, greater than about 80% of the compounds or materials are devoid of crosslinking bridges, with greater than about 90% being still more preferred. In particularly preferred embodiments, greater than about 95% of the compounds or materials are devoid of crosslinking bridges. If desired, the substantially non-crosslinked compounds or materials may be completely non-crosslinked (i.e., about 100% of the compounds or materials are devoid of crosslinking bridges).

[0065] When a polymer is used as the stabilizing agent in the formulation of the invention, the polymer, e.g. branched block copolymer, is generally formulated with the active drug, e.g. SN-38, in a weight ratio from about 0.1% by weight polymer up to about 99% by weight polymer, with the drug ranging from between 99.9% by weight to about 0.1% by weight. Preferably, the polymer ranges from about 50 to about 95% by weight. More preferably, the polymer ranges from about 70 to about 90% by weight relative to the amount of drug. Most preferably, the polymer is about 85 to 90% by weight relative to the drug.

[0066] Examples of suitable polymeric stabilizing agents include, but are not limited to, polyethylene glycol, polypropylene glycol, polyvinyl alcohol, polyvinyl pyrrolidone, polylactide, poly(lactide-co-glycolide), polysorbate, polyethylene oxide, polycaprolactone, polypropylene oxide, poly(ethylene oxide-co-propylene oxide), poly(oxyethylated) glycerol, poly(oxyethylated) sorbitol, poly(oxyethylated) glucose), and derivatives, mixtures, and copolymers thereof. Examples of suitable derivatives include those in which one or more C-H bonds, e.g., in alkylene linking groups, are replaced with C-F bonds, such that the polymers are fluorinated or even perfluorinated.

[0067] In one embodiment, the polymer comprises repeating alkylene units, wherein each alkylene unit optionally contains from one to three heteroatoms selected from -O-, -N(R)- or $-S(O)_n-$, where R is hydrogen or alkyl and n is 0, 1 or 2. Preferably, the number of alkylene units are 2,3,4, or 5 units. The polymers may be linear (e.g., the type AB random sequence of units or AB block where two or more units of A are linked to two or more units of B, type ABA, ABABA or ABCBA alternating units or blocks, and the like), branched (e.g., the type A_nB or BA_nC, and the like, where A is at least n-valent, and n is an integer ranging from about 3 to about 50, and all combinations and subcombinations of ranges and specific integers therein or multiple A's extending from one B), with branched polymers being preferred. When a branched polymer is employed, particularly when the branched polymer includes an inner, more hydrophobic core region and an outer, more hydrophilic region, the resulting delivery system may be in the form of a nanoparticle. An exemplary illustration of such a delivery system occurs when a branched block copolymer structure binds a plurality of molecules of an active agent, for example, SN-38. In another embodiment, the branched polymer used includes an inner more hydrophilic core region and an outer, more hydrophobic region, the resulting delivery system is in the form of a nanoparticle. Once again, this branched block copolymer binds a plurality of molecules of an active agent, for example,

SN-38. When branched polymers are used, they contain between about 4 and 40 arms, more preferably between 4 and 10 arms, more preferably between 4 and 8 arms, and most preferably 4 arms. When branched polymers are used, these preferably contain but are not limited to one or a combination of two or more of the following polymers; polyethylene glycol, polypropylene glycol, polycaprolactone, polylactide, polyglycolide, and, polylactide-co-glycolide.

[0068] As discussed above, one preferred polymer for use as a stabilizing agent in the present formulations is polyethylene glycol (PEG) or a copolymer thereof, e.g., polyethylene glycol containing some fraction of other monomer units (e.g., other alkylene oxide segments such as propylene oxide), with polyethylene glycol itself most preferred. The polyethylene glycol used may be either linear or a branched PEG. In certain embodiments, the polymer may be covalently associated with a lipid, such as a phospholipid moiety in which the hydrophobic chains of the phospholipids may tend to associate in an aqueous medium. Combinations of different types of PEG (e.g., branched PEG and linear PEG, branched PEG and phospholipid-conjugated linear PEG, etc.) may also be employed. In other embodiments the polymer may be covalently associated with a fatty acid with a carbon chain length of 6 to 22 carbons.

[0069] With respect to branched polymers, the molecular weight of the entire branched polymer may range from about 2000 to 1,000,000 daltons, preferably from about 5000 to 100,000 daltons, more preferably from about 10,000 to 60,000 daltons, and still more preferably about 20,000 daltons. Preferably, each arm has the same unit size of polymer, such as PEG, e.g., about 2500 daltons each for an 8-armed PEG. In the case of a branched copolymer, the various percentages of the hydrophobic and hydrophilic monomers or blocks in each arm may vary. For example, with an 8 arm branched copolymer of polypropylene glycol (PPG) and PEG, when 50% is PPG and 50% is PEG, both the PPG segment and the PEG segment will have a molecular weight about 1250 daltons, with the PEG forming the outer portion of the arm.

[0070] Branched PEG molecules will generally although not necessarily have a molecular weight in the range of approximately 1,000 to 600,000 daltons, more typically in the range of approximately 2,000 to 100,000 daltons, preferably in the range of approximately 5,000 to 40,000 daltons. Branched PEG is commercially available, such as from Nippon Oil and Fat (NOF Corporation, Tokyo, Japan) and from Shearwater Polymers (Huntsville, Alabama), or

may be readily synthesized by polymerizing lower molecular weight linear PEG molecules (i.e., PEG 2000 or smaller) functionalized at one or both termini with a reactive group. For example, branched PEG can be synthesized by solution polymerization of lower molecular weight PEG acrylates (i.e., PEG molecules in which a terminal hydroxyl group is replaced by an acrylate functionality -O-(CO)-CH=CH_2) or methacrylates (similarly, PEG molecules in which a hydroxyl group is replaced by a methacrylate functionality -O-(CO)-C(CH_3)=CH_2) in the presence of a free radical polymerization initiator such as 2,2'-azobisisobutyronitrile (AIBN). If desired, mixtures of PEG monoacrylates or monomethacrylates having different molecular weights can be used in order to synthesize a branched polymer having "branches" or "arms" of differing lengths. Branched PEGs have 2 or more arms but may have as many as 1,000 arms. The branched PEGs herein preferably have about 4 to 40 arms, more preferably about 4 to 10 arms, and most preferably about 4 to 8 arms. Higher molecular weight, highly branched PEG, e.g., branched PEG having a molecular weight of greater than about 10,000 and at least about 1 arm (i.e., one branch point) per 5,000 daltons, will sometimes be referred to herein as "dendrimeric" PEG. Dendrimeric PEG may preferably be formed by reaction of a hydroxyl-substituted amine, such as triethanolamine, with lower molecular weight PEG that may be linear, branched or star, to form a molecular lattice that may serve as the spatially stabilized matrix for delivery of an entrapped active agent. Dendrimeric structures, including dendrimeric PEG, are described, *inter alia*, by Liu et al. (1999) *PSTT* 2(10):393-401.

[0071] Star molecules of PEG are available commercially (e.g., from Shearwater Polymers, Huntsville, Ala.) or may be readily synthesized using free radical polymerization techniques as described, for example, by Gnanou et al. (1988) Makromol. Chem. 189:2885-2892 and U.S. Pat. No. 5,648,506 to Desai et al., the disclosures of which are hereby incorporated herein by reference, in their entireties. Star PEG typically has a central core of pentaerythritol, or glycerol. Preferred molecular weights for star molecules of PEG may be from about 1000 to 500,000 Daltons, with molecular weights of about 10,000 to 200,000 being preferred. The active agent may be associated with the branches and/or arms of the matrix, and/or may be associated with the core portions of the matrix structures.

[0072] The polymers employed in the present matrices may be selected so as to achieve the desired chemical environment to which the active agent may be exposed. Specifically, in the case, for example, of star polymers, the inner core region may generally be relatively more hydrophobic, and the arms or branches may generally be more hydrophilic. Alternatively, the

inner core region may generally be relatively more hydrophilic, and the arms or branches may generally be more hydrophobic. It should be understood, however, that the chemical structures of the core, arms and branches of the polymer may be selected, as desired, so as to modify or alter the generally hydrophobic nature of the core (for example, by increasing or decreasing the core's hydrophobicity) and the generally hydrophilic nature of the arms and/or branches (for example, by increasing or decreasing the hydrophilicity of the arms and/or branches).

[0073] The number of "branches" or "arms" in star polymers may range from about 3 to 50, with from about 3 to 30 being preferred, and from about 3 to 12 branches or arms being more preferred. Even more preferably, the star polymers contain from about 4 to 8 branches or arms, with either about 4 arms or about 8 arms being still more preferred, and about 4 arms being particularly preferred. Preferred branched polymers may contain from about 3 to 1000 branches or arms (and all combinations and subcombinations of ranges and specific numbers of branches or arms therein). As noted above, preferred branched polymers may have from about 4 to 40 branches or arms, even more preferably from about 4 to 10 branches or arms, and still more preferably from about 4 to 8 branches or arms.

[0074] In accordance with certain preferred embodiments, the polymer, whether linear or branched, may be selected from the group consisting of a polyalkylene oxide, polyalkyleneimine, polyalkylene amine, polyalkene sulfide, polyalkylene sulfonate, polyalkylene sulfone, poly(alkylenesulfonylalkyleneimine), polycaprolactone, polylactide, polyglycolide, and copolymers thereof.

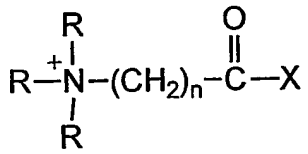
[0075] In one embodiment of the present invention, the branched polymer comprises a block copolymer. The block copolymer may be a mixture of hydrophobic and hydrophilic blocks, but preferentially with hydrolyzable bonds. The block copolymer may arise from a central core of, for example, a sugar molecule, a polysaccharide or a frame polymer. In a preferred form, the block copolymer preferably includes a central core from which radiate about 3 to 12 arms, with from about 4 to 8 arms preferred.

[0076] In one embodiment, each arm may comprise a block copolymer with an inner, more hydrophobic block and an outer, more hydrophilic block. In preferred embodiments, the inner block may comprise polypropylene oxide (PPO), polylactide (PLA), polylactide-coglycolide (PLGA) or b-polycaprolactone, and the outer block comprises polyethylene glycol, PEG-PPO, PEG-PLA, PEG-PLGA, and PEG-b-polycaprolactone, respectively. Also

in preferred embodiments, the targeting ligands may be attached to the outermost portion of the arms. In another embodiment, each arm may comprise a block copolymer with an inner, more hydrophilic block and an outer, more hydrophobic block, also referred to as reverse block copolymers.

[0077] In certain embodiments, the polymer may have a multivalent core structure from which extend arms comprising linear or branched polymers. The cores may preferably be polyhydroxylated monomers such as sugars, sugar alcohols, polyaliphatic alcohols and the like. Preferred among such core structures are triethanolamine, which contains three hydroxyl moieties; and neopentanol and polyerythritol, which contain four hydroxy moieties that may be derivatized to afford the various arms or branches. Sugar alcohols such as glycerol, mannitol and sorbitol may also be similarly derivatized.

[0078] The polymer may be modified in one or more ways. For drugs that are ionized at physiological pH, charged groups may be inserted into the hydrophilic polymer in order to modify the sustained release profile of the formulation. To reduce the rate of drug release and thereby provide sustained delivery over a longer time period, negatively charged groups such as phosphates and carboxylates are used for cationic drugs, whereas positively charged groups such as quaternary ammonium groups are used in combination with anionic drugs. To insert such groups, a terminal hydroxyl group of a hydrophilic polymer such as PEG may be converted to a carboxylic acid or phosphate moiety by using a mild oxidizing agent such as chromic (VI) acid, nitric acid, or potassium permanganate. A preferred oxidizing agent is molecular oxygen used in conjunction with a platinum catalyst. Introduction of phosphate groups may be carried out using a phosphorylating reagent such as phosphorous oxychloride ($POCl_3$). Terminal quaternary ammonium salts may be synthesized, for example, by reaction with a moiety such as



wherein R is H or lower alkyl (e.g., methyl or ethyl), n is typically 1 to 4, and X is an activating group such as Br, Cl, I, or an -NHS ester. If desired, such charged polymers may be used to form higher molecular weight aggregates by reaction with a polyvalent counter ion.

[0079] Other possible modifications to the hydrophilic polymer include, but are not limited to, the following. A terminal hydroxyl group of a PEG molecule may be replaced by

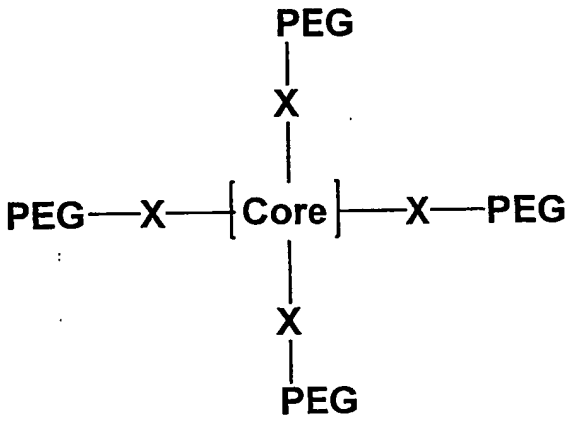
a thiol group using conventional means, e.g., reacting hydroxyl-containing PEG with a sulfur-containing amino acid such as cysteine, using a protected and activated amino acid. The resulting polymer ("PEG-SH") is also commercially available, for example from Shearwater Polymers. Alternatively, a mono(lower alkoxy)-substituted PEG such as monomethoxy polyethylene glycol (MPEG) may be used instead of polyethylene glycol *per se*, so that the polymer terminates with a lower alkoxy substituent (such as a methoxy group) rather than with a hydroxyl group. Similarly, an amino substituted polymer such as PEG amine, may be used in lieu of the corresponding non-substituted polymer, e.g., PEG, so that a terminal amine moiety ($-NH_2$) may be present rather than a terminal hydroxyl group.

[0080] In addition, as discussed above, the polymer may contain two or more types of monomers, as in a copolymer wherein propylene oxide ($-CH_2CH_2CH_2O-$), lactide ($-OCH(CH_3)CO-$), glycolide ($-OCH_2CO-$), or caprolactone groups ($-O(CH_2)_5CO-$), have been substituted for some fraction of ethylene oxide groups ($-CH_2CH_2O-$) in polyethylene glycol, for example, four-arm poly(ethylene oxide-b-lactide) L form or four-arm poly (ethylene oxide-b-caprolactone) (branched PEG-b-polycaprolactone). Incorporating these groups may tend to increase the stability of the spatially stabilized matrix that entraps the drug, thus decreasing the rate at which the drug may be released in the body. The more hydrophobic the drug and the larger the fraction of propylene oxide or other hydrophobic blocks, the slower the drug release rate will be. Generally speaking therefore, by increasing the hydrophobicity of the camptothecin analog complex and the fraction of hydrophobic blocks may result in a slower rate of release of the agent from the matrix.

[0081] The polymer may also contain hydrolyzable linkages to enable hydrolytic degradation within the body, and thus facilitate drug release from the polymeric matrix. Suitable hydrolyzable linkages include any intramolecular bonds that can be cleaved by hydrolysis, typically in the presence of acid or base. Examples of hydrolyzable linkages include, but are not limited to, those disclosed in WO 99/22770 to Harris, such as carboxylate esters, phosphate esters, acetals, imines, ortho esters, and amides.

[0082] Other suitable hydrolyzable linkages include, for example, enol ethers, diketene acetals, ketals, anhydrides, and cyclic diketenes. Formation of such hydrolyzable linkages within the hydrophilic polymer is conducted using routine chemistry known to those skilled in the art of organic synthesis and/or described in the pertinent texts and literature. For example, carboxylate linkages may be synthesized by reaction of a carboxylic acid with an

alcohol, phosphate ester linkages may be synthesized by reaction of a phosphate group with an alcohol, acetal linkages may be synthesized by reaction of an aldehyde and an alcohol, and the like. Thus, polyethylene glycol containing hydrolyzable linkages "X" might have the structure -PEG-X-PEG- or alternatively might be a matrix having the structure



where the core is a hydrophobic molecule such as pentaerythritol. Such polymers may be synthesized by reaction of various -PEG-Y molecules with -Core-Z or PEG-Z molecules wherein Z and Y represent groups located at the terminus of individual PEG molecules and are capable of reacting with each other to form the hydrolyzable linkage X.

[0083] Accordingly, it will be appreciated that the rate of drug release from the stabilized camptothecin analog matrix can be controlled by adjusting the degree of branching of the polymer, by incorporating different types of monomer units in the polymer structure, by functionalizing the hydrophilic polymer with different terminal species (which may or may not be charged), and/or by varying the density of hydrolyzable linkages present within the polymeric structure.

[0084] As noted above, depending on the particular polymer employed, the polymers may be relatively more hydrophilic or relatively more hydrophobic. Examples of suitable, relatively more hydrophilic polymers include, but are not limited to, polyethylene glycol, polypropylene glycol, branched polyethylene imine, polyvinyl pyrrolidone, polylactide, poly(lactide-co-glycolide), polysorbate, polyethylene oxide, poly(ethylene oxide-co-propylene oxide), poly(oxyethylated) glycerol, poly(oxyethylated) sorbitol, poly(oxyethylated glucose), polymethyloxazoline, polyethyloxazoline, polyhydroxyethyloxazoline, polyhydroxypropyloxazoline, polyvinyl alcohol, poly(hydroxyalkylcarboxylic acid), polyhydroxyethyl acrylic acid, polyhydroxypropyl methacrylic acid, polyhydroxyvalerate,

polyhydroxybutyrate, polyoxazolidine, polyaspartamide, polysialic acid, and derivatives, mixtures and copolymers thereof.

[0085] Examples of suitable, relatively more hydrophobic polymers include linear polypropylene imine, polyethylene sulfide, polylactide, polyglycolide, polypropylene sulfide, polyethylenesulfonate, polypropylenesulfonate, polyethylene sulfone, polyethylenesulfonylethyleneimine, polycaprolactone, polypropylene oxide, polyvinylmethylether, polyhydroxyethyl acrylate, polyhydroxypropyl methacrylate, polyphosphazene and derivatives, mixtures and copolymers thereof.

[0086] Preferred among the foregoing polymers for use in the present compositions are polyethylene glycol (PEG), polypropylene glycol (PPG), and copolymers of PEG and PPG, or PEG and/or PPG containing some fraction of other monomer units (e.g., other alkylene oxide segments such as propylene oxide). Other preferred copolymers are branched copolymers containing PEG and Caprolactone, PEG and lactide, and PEG and [lactide-co-glycolide] where the core is comprised of either the more hydrophilic or the more hydrophobic polymer. Another particularly preferred copolymer is a branched polymer of PEG and PPG, particularly wherein the PPG units comprise the innermost portion of the structure and the PEG units comprise the outer portions of the arms of the branched structure. Also preferred among the foregoing polymers are polysorbates, particularly polysorbate 80 (commercially available as TWEEN®80), sorbitan mono-9-octadecanoate poly(oxy-1,2-ethanediyl) derivatives.

[0087] As illustrated above, the branched PEG molecule may be modified to have a hydrophobic core. For example, if the central core is pentaerythritol, the innermost arms bound to the pentaerythritol may comprise a polymer more hydrophobic than PEG. Useful polymers to accomplish this include polypropylene glycol and polybutylene glycol. Useful monomers for constructing the inner, hydrophobic core structures of the arms include propylene oxide, butylene oxide, and copolymers of the two; and lactic acid and copolymers of lactic acid with glycolide (polylactide-co-glycolide and copolymers of the foregoing with polyethylene glycol). The preferred materials for constructing an inner hydrophobic core include polypropylene glycol and copolymers of propylene oxide with ethylene oxide. Useful polymers for constructing the outer, peripheral parts of the arms include polyethylene glycol, polycaprolactone, polylactide, and poly[lactide-co-glycolide], polysialic acid, and other hydrophilic polymers, with PEG most preferred. It is possible that a fraction of the

monomers in the outer portion of a given arm of the carrier molecule may be replaced with PEG, but in this case, there will be substantially more of the hydrophilic monomer (e.g., ethylene oxide) than the hydrophobic monomer (e.g., propylene oxide).

[0088] The relative proportion of hydrophobic polymer within the branched polymer may vary from about 10 wt% to about 99 wt% on a weight/weight ratio, preferably from about 25 wt% to about 95 wt%. When more hydrophobic polymer is used, the drug loading capacity of the branched molecule may be increased for hydrophobic drugs. A most preferred ratio is about 10 wt% of hydrophobic polymer, e.g., polypropylene glycol, and 90 wt% of hydrophilic polymer (e.g., PEG) in the outer arms.

[0089] The branched molecules comprising a hydrophobic core and peripheral hydrophilic arms are thought to have a number of advantages for drug delivery. The hydrophobic core may better stabilize hydrophobic drugs within the branched molecule and, as the drug is stabilized within the core, the free arms of the PEG may be better able to maintain a random state in which the PEG molecules move freely within solution. The outer, hydrophilic PEG layer may act as a steric barrier, inhibiting or decreasing the aggregation of individual branched molecules into particles. Additionally, in instances when targeting ligands are attached to the termini of the peripheral hydrophilic arms, targeting is facilitated by the unencumbered and exposed nature of the outer PEG arms. As will be discussed further on, a wide variety of targeting ligands can be covalently bound to the free ends of the PEG. The hydrophobic and hydrophilic components of the arms may be linked together by a variety of different linkers. Such linkers include ethers, amides, esters, carbamates, thioesters, and disulfide bonds. In general, the linker employed is used to attain the desired drug delivery properties of the pharmaceutical formulation. Metabolizable bonds can be selected to improve excretion of the carrier molecule as well as to improve drug release.

[0090] The branched molecules comprising a hydrophilic core and peripheral hydrophobic arms are also thought to have a number of advantages for drug delivery. The hydrophilic core may better solubilize hydrophobic drugs by forming a spatially stabilized matrix in which the hydrophobic moieties serve to sequester the drug and the hydrophilic moieties interact with the aqueous solution. Additionally, in instances when targeting ligands are attached to the termini of the peripheral hydrophobic arms, targeting is facilitated by the unencumbered and exposed nature of the outer polymer arms. As will be discussed further on, a wide variety of targeting ligands can be covalently bound to the free ends of the

hydrophobic polymers. The hydrophilic and hydrophobic components of the arms may be linked together by a variety of different linkers. Such linkers include ethers, amides, esters, carbamates, thioesters, and disulfide bonds. In general, the linker employed is used to attain the desired drug delivery properties of the pharmaceutical formulation. Metabolizable bonds can be selected to improve excretion of the carrier molecule as well as to improve drug release.

[0091] As previously mentioned, when branched PEG polymers are used as the stabilizing agent, the free ends of the branches can be substituted with one or more targeting ligands per carrier molecule. More than one kind of targeting ligand may be bound to each carrier molecule to facilitate binding to a target cell bearing more than one kind of receptor. A wide variety of ligands may be used in this regard. Exemplary targeting ligands include, for example, proteins, peptides, polypeptides, antibodies, antibody fragments, glycoproteins, carbohydrates, hormones, hormone analogs, lectins, amino acids, sugars, saccharides, vitamins, steroids, steroid analogs, enzyme cofactors, and genetic material. Suitable targeting ligands and methods of synthesizing and attaching such ligands are also described in WO 01/49268 to Unger et al.

B. LIPIDS

[0092] The stabilizing agent can also be a lipid, which includes phospholipids and lecithins, where the phospholipid can be a natural phospholipid, a chemically or enzymatically modified phospholipid, or a synthetic phospholipid. Examples of suitable lipids include, but are not limited to, phosphatidylglycerol, phosphatidic acid, phosphatidylserine, phosphatidylinositol, cerebrosides, gangliosides, sphingosines, cardiolipin, and sulfatides.

[0093] Other suitable phospholipids include diacyl phospholipids such as diacyl derivatives of phophatidylcholine (diacyl phosphatidylcholines), phosphatidylethanolamine (diacyl phosphatidylethanolamines), phosphatidylserine (diacyl phosphatidylserines), phosphatidylglycerol (phosphorylated diacylglycerides), phosphatidylinositol (diacyl phosphatidylinositols and phosphatidic acid (diacyl phosphatidic acids), and combinations thereof. The fatty acyl chain may be from 10 to 22 carbons in length and may be saturated, monounsaturated, or polyunsaturated. The fatty acid at the 1 and 2 positions may be mixed or the same in the acylglyceryl moieties. Preferred saturated fatty acyl moieties include lauryl,

myristyl, palmityl, stearyl, or higher chain derivatives; preferred unsaturated acyl moieties include oleyl chains. A given phospholipid may contain two identical chains, as in DOPE (dioleylphosphatidylethanolamine), or mixed chains as in POPE (1-palmitoyl-2-oleylphosphatidylethanolamine).

[0094] Exemplary diacyl phosphatidylcholines include, by way of example, palmitoyloleoyl phosphatidylcholine (POPC), dioleoyl phosphatidylcholine (DOPC), dilauroyl phosphatidylcholine (DLPC), dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC), and combinations thereof. Exemplary diacyl phosphatidylethanolamines include, by way of example, dipalmitoyl phosphatidylethanolamine (DPPE), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), dioleylphosphatidylethanolamine (DOPE), and combinations thereof. Exemplary phosphorylated diacylglycerides include, for example, dioleoyl phosphatidylglycerol (DOPG), palmitoyloleyl phosphatidylglycerol (POPG), and combinations thereof. POPG is a particularly preferred lipid.

[0095] When an lipid is employed as the stabilizing agent in the formulation, the amount of lipid may range from about 0.1% by weight up to about 99% of the formulation. More preferably the lipid will range from about 1% to about 90% by weight and still more preferably from about 2% to about 50% by weight. Preferred ratios of lipid to drug weight ratio are less than 5:1, more preferably less than 3:1, most preferably less than 1:1.

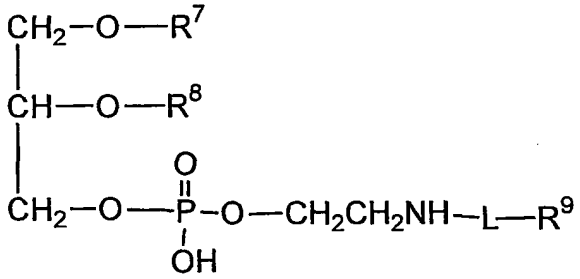
C. POLYMER-LIPID CONJUGATES

[0096] The polymers employed in the present compositions may also be linked or conjugated to a lipid, preferably a phospholipid, to provide a polymer-lipid conjugate, as in the case, for example, of PEG-phospholipid conjugates. The polyethylene glycol in the PEGylated phospholipids may be branched or linear, and may be derivatized with amino, carboxyl, acyl, or sulfonyl ends. Conjugates of linear PEG and phospholipids, if used, will generally although not necessarily employ PEG have a molecular weight in the range of about 100 to 50,000 daltons, preferably about 350 to 40,000 daltons, more preferably about 350-7000, and even more preferably about 750-5000 daltons. It will be appreciated by those skilled in the art that the aforementioned molecular weight ranges correspond to a polymer containing approximately 2 to 1,000 ethylene oxide units, preferably about 8 to 800 ethylene oxide units. The phospholipid moiety that is conjugated to the PEG may be anionic, neutral,

or cationic, of naturally occurring or synthetic origin, and normally comprises a diacyl phosphatidylcholine, a diacyl phosphatidylethanolamine, a diacyl phosphatidylserine, a diacyl phosphatidylinositol, a diacyl phosphatidylglycerol, or a diacyl phosphatidic acid, wherein each acyl moiety can be saturated or unsaturated and will generally be in the range of about 10 to 22 carbon atoms in length.

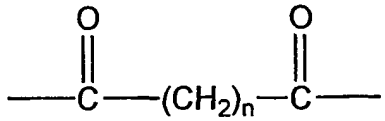
[0097] Exemplary PEGylated phospholipids include, by way of example, diacyl lipid-PEG conjugates such as DPPE-PEG, DOPE-PEG, POPE-PEG, where the PEG length can vary so as to provide for a PEG molecular weight of 2kDa, 5kDa, 10kDa, and greater. In addition, PEG can be conjugated to a fatty acid, for example as a Myrj compound, e.g., Myrj 52.

[0098] Preferred compounds are polymer-conjugated diacyl phosphatidyl-ethanolamines having the structure of formula (III)



(III)

wherein R^7 and R^8 are the acyl groups, R^9 represents the hydrophilic polymer, e.g., a polyalkylene oxide moiety such as PEG, poly(ethylene oxide), poly(propylene oxide), poly(ethylene oxide-co-propylene oxide), or the like (for linear PEG, R^9 is -O-$(CH_2CH_2O)_n$-H), and L is an organic linking moiety such as a carbamate, an ester, an amide, an imine, an amine, or a diketone having the structure of formula (IV)



(IV)

wherein n is 1, 2, 3, or 4. Preferred unsaturated acyl moieties are esters formed from oleic and linoleic acids, and preferred saturated acyl moieties are palmitate, myristate, and stearate. Particularly preferred phospholipids for conjugation to linear or branched PEG herein are dipalmitoyl phosphatidylethanolamine (DPPE), dioleoyl phosphatidylethanolamine (DOPE), and 1-palmitoyl-2-oleyl phosphatidylethanolamine (POPE).

[0099] The conjugates may be synthesized using art-known methods such as described, for example, in U.S. Patent No. 4,534,899 to Sears. That is, synthesis of a PEG-phospholipid conjugate or a conjugate of a phospholipid and an alternative hydrophilic polymer may be carried out by activating the polymer to prepare an activated derivative thereof, which has a functional group suitable for reaction with an alcohol, a phosphate group, a carboxylic acid, an amino group, or the like. For example, a polyalkylene oxide such as PEG may be activated by the addition of a cyclic polyacid, particularly an anhydride such as succinic or glutaric anhydride (ultimately resulting in the linker of formula (II) wherein n is 2 or 3, respectively). The activated polymer may then be covalently coupled to the selected phosphatidylalkanolamine, such as phosphatidylethanolamine, to give the desired conjugate.

D. MIXTURES

[00100] In another embodiment, the stabilizing agent contains a mixture of polymeric stabilizing agents and lipidic stabilizing agents. For example, a particularly preferred formulation might contain about 2 parts drug, 1 part poloxamer, and 8 parts by weight of phosphatidylglycerol, wherein the drug is SN-38. Preferred ranges for the ratio of drug to lipid to polymeric component when combinations of stabilizing agents are used range between approximately 1:2:1 to approximately 1:20:5, most preferably from approximately 1:5:1 to approximately 1:10:2. In embodiments where lipids and polymers are both used as the stabilizing agent, the polymeric component of the stabilizing agent is generally added during rehydration, as will be discussed below.

[00101] Secondary stabilizing agents may also be added to the formulation and are useful for reducing particle size. Preferably, the secondary stabilizing agent acts to stabilize the surface of the complex by virtue of a combination of hydrophilic and hydrophobic interactions. Thus, it is preferred that the secondary stabilizing agent polymer contains both hydrophilic and hydrophobic groups or domains, thus allowing the combination of interactions to occur. It is also preferred that the secondary stabilizing agent contain a sufficient amount of hydrophilic surface area so that post-stabilization nanoparticles remain suspended within an aqueous solution and avoid clumping.

[00102] An exemplary secondary stabilizing agent is a polymer having a molecular weight ranging from about 400 daltons to about 400,000 daltons, more preferably from about 1,000 daltons to about 200,000 daltons, and still more preferably from about 3,000 daltons to about

100,000 daltons. The secondary stabilizing agent may be derived from natural, recombinant, synthetic, or semisynthetic sources. Most preferably, the secondary stabilizing agent will be a lipid, protein or a peptide. Useful preferred lipids include phosphatidylglycerols, phosphatidylserines, and phosphatidylinositols. Useful preferred proteins include albumin, collagen, fibrin, immunoglobulins, hemoglobin, vascular endothelial growth factor, vascular permeability factor, epidermal growth factor, fibroblast growth factor, fibronectin, vitronectin, and cytokines such as interleukins (e.g., IL-3 and IL-12).

[00103] Suitable secondary stabilizing proteins include, but are not limited to: serum proteins, i.e., albumin (especially recombinant and defatted), amylins, atrial natriuretic peptides, endothelins and endothelin inhibitors, urokinase, streptokinase, staphylokinase, vasoactive intestinal peptide, HDL, LDL, VLDL, etc.; agglutination (antihemophilia) factors (e.g., Factor VIII, Factor IX, and subtypes thereof), decorsin, serum thymic factor, etc.; peptide hormones, e.g., ACTH, FSH, LH, thyroxin, insulin, vasopressin, bradykinin and bradykinin potentiators, HGH, CRF (corticotropin releasing factor), oxytocin, gastrins, LH-RH, MSH (melanocyte stimulating hormone) and MSH releasing factor; parathyroid hormones and analogs; pituitary adenylate cyclase activating polypeptide; secretins; thyrotropin releasing hormone, etc.; structural proteins, e.g., collagens, amyloid proteins, brain natriuretic peptides, elafin, fibronectin and fibronectin fragments, laminin, sarafotoxins, etc.; growth factors, e.g., nerve growth factor, platelet derived growth factor, epidermal growth factor, vascular endothelial growth factor, tumor necrosis factor, CINC- I (cytokine-induced neutrophil chemoattractant), growth hormone releasing factor, liver cell growth factor, midkines, neurokinins, neuromedins, etc.; metabolic potentiators, e.g., erythropoietin, adrenomedullin and adrenomedullin antagonists, o-agatoxin TK, agelenin, angiotensins, calcicludine, calciseptine, calcitonin and calcitonin antagonists, calmodulin, charybdotoxin, chlorotoxin, conotoxins, endorphins, neo-endorphins, glucagon and variants, guanylins, iberiotoxin, kaliotoxin, margatoxin, mast cell degranulating peptide, neurotensins, pancreastatins, PLTX-11, scylotoxin, ATPase inhibitors, somatostatins, somatomedin, uroguanylin, etc.; nuclear binding proteins, e.g., histones, spermine, spermidine, nuclear localization sequences, telomerase, etc.; enzymes, e.g., cholecystokinin, cathepsins, etc.; antivirals, i.e., IFN-α, IFN-β, IFN-γ, virus replication inhibiting peptide, etc.; immunoglobulins, i.e., IgA, IgD, IgE, IgG, IgH, and subtypes; and miscellaneous proteins such as apamin, bombesin, casomorphins, conantokins, defensin-1, dynorphins, enkephalins,

galanins, magainin, nociceptin, osteocalcins, substance P, xenin, etc. While not wishing to be limited to the preceding examples, one of skill in the art will recognize that the examples given may be used individually or in combination.

[00104] The secondary stabilizing protein may also serve as a targeting agent or binding ligand to direct the nanoparticles and drugs therein to a certain site. One preferred protein is albumin, in particular human serum albumin, and even more preferably recombinant derived human albumin. Another preferred protein is defatted albumin, either native or recombinant. For veterinary applications, the albumin is preferably from the patient's species. The stabilizing albumin is generally added to the nanoparticles at an effective stabilizing concentration, generally in the range of about 0.001 to about 10% w/v, preferably in the range of about 0.01 to about 5%, more preferably in the range of about 0.1 to about 2.5%, and most preferably in the range of about 0.25% to about 1.5%. Note that more than one protein may be used to stabilize the nanoparticles. For example, the particles may be formulated with about 1.0% w/v albumin and about 0.1% w/v EGF. In this case, the EGF serves as a targeting ligand to help the nanoparticle bind to tissues with increased expression of the EGF receptor.

[00105] The protein may be naturally occurring, a protein fragment (e.g., a fragment of the gamma-carboxy terminus of fibrinogen), or chemically modified. For example, albumin or other proteins may be modified with one or more hydrophilic or targeting moieties. The protein, for example, may be modified by binding one or more PEG residues per protein molecule, typically between 1 and 100 PEG residues per protein molecule, but more preferably between 1 and 10 PEG residues. For example, mono or bifunctional PEG groups may be coupled to the protein through linkages such as ethers or biodegradable bonds such as esters, amides, carbamates, thioesters, disulfides, thiocarbamates, phosphate esters, and phosphoamides. The resulting "PEGylated" protein enables the protein to stabilize the surface of the nanoparticle while the PEG groups help to protect the nanoparticle surface from nonspecific interaction with serum proteins. In this manner, the "PEGylated" proteins increase the serum half-lives of the nanoparticles.

[00106] In addition to the materials enumerated above, the secondary stabilizing polymers may be a natural polymer, such as: cellulose and dextran; semi-synthetic cellulose derivatives such as methylcellulose and carboxymethylcellulose; and synthetic polymers such as polvinylalcohol polyvinylpyrrolidone and copolymers containing PEG and a second polymer such as polypropylene glycol (PPG) (e.g. those available under the Pluronic trademark);

polycaprolactone, polylactide, and poly[lactide-co-glycolide]. Synthetic polymers such as the PLURONICS®, i.e. copolymers of PEG and PPG, may be incorporated into mixtures of secondary stabilizing agents, e.g., with albumin. Preferred block copolymers include, but are not limited to, polyethylene glycol-N-carboxyanhydride of 6-(benzyloxycarbonyl)-l-lysine, polyethylene glycol-poly-l-lysine, and polyethylene glycol-polyaspartic acid. Methods for synthesizing the above copolymers are described in detail by Harada et al (1995) *Macromolecules* 28:5294-5299. One of skill in the art will readily recognize that the same synthetic methods can be used to substitute polypropylene glycols for PEG to make the PPG block copolymer analogs of the above.

IV. OTHER COMPONENTS OF THE FORMULATION

[00107] Other moieties may be incorporated into the present formulations as excipients in order to reduce the particle size of the stabilized camptothecin analog matrix. For intravenous administration in particular, particle size is critical, and is generally in the range of about 1 nm to 1000 nm, preferably in the range of about 100 nm to 900 nm, and most preferably in the range of about 200 nm to 800 nm (the values given are number-weighted average).

[00108] Compounds other than the stabilizing agents are also useful for reducing particle size; these other compounds include, but are not limited to, cholic acids, cholic acid salts, saccharides (such as sorbitol, sucrose, trehalose, mannitol, and inositol), polyhydroxyalcohols (such as glycerol), and liquid polyethylene glycols (i.e., PEG having a molecular weight less than about 1,000 daltons). The formulations of the invention can also contain pharmaceutically acceptable auxiliary agents as required in order to approximate physiological conditions; such auxiliary agents include pH adjusting and buffering agents (e.g., citrate and phosphate buffers), tonicity adjusting agents, and the like. Lipid-protecting agents that serve to minimize free radical and peroxidative damage upon storage may also be advantageous. Suitable lipid protective agents include alpha-tocopherol, ethylenediaminetetraacetic acid (EDTA) and water-soluble, iron-specific chelators such as deferoxamine. Additionally, for lyophilized compositions that are to be hydrated prior to use, it may be desirable to include one or more cryoprotectants or antiflocculants in order to facilitate rehydration and formation of a substantially homogeneous suspension. For compositions that are to be stored in liquid form, it is preferred that one or more conventional

antibacterial agents be included. Still other additives that may be incorporated into the present formulations include radioactive or fluorescent markers useful for imaging purposes. Radioactive markers include, for example, technetium-99 and indium-111, while an exemplary fluorescent marker is fluorescein. The excipients can be included in an amount up to about 50 wt% of the formulation, but preferably represent less than about 10 wt% of the formulation.

[00109] Generally, any additional components to the formulation are added to the complex in an aqueous medium. The complex and additional components are then subjected to a mechanical dispersal process that helps to break the complex into nanoparticles stabilized by the stabilizing agent and incorporating the additional components. Useful mechanical dispersal processes include shaking, agitation (e.g., vortexing), sonication, extrusion under pressure, microfluidization, microemulsification, and high speed blending.

C. MANUFACTURE AND STORAGE

[00110] The formulations of the invention are manufactured using standard techniques and reagents known to those skilled in the art of pharmaceutical formulation and drug delivery and/or described in the pertinent texts and literature. See *Remington: The Science and Practice of Pharmacy*, 19th Ed. (Easton, PA: Mack Publishing Co., 1995), which discloses conventional methods of preparing pharmaceutical compositions that may be used as described or modified to prepare pharmaceutical formulations of the invention. In one embodiment, the stabilizing agent and camptothecin analog are mixed together in an organic solvent or solvent system such as isopropanol, t-butanol, DMSO/t-butanol, benzene/methanol, ethanol, or an alternative suitable solvent as will be apparent to those of skill in the art, and then lyophilized. Other embodiments are set forth in the Examples.

[00111] The lyophilized mixture is then rehydrated with a rehydration solution that may contain an additional component of the stabilizing agent. In embodiments where the stabilizing agent comprises a lipidic stabilizing agent and a polymeric stabilizing agent, the lipidic component is first mixed with the camptothecin analog and freeze-dried, and the polymeric component is added to the formulation during the rehydration step. Additional components such as secondary stabilizing agents, e.g., proteins, excipients, and targeting ligands, may also be incorporated into the formulation during rehydration.

[00112] It is significant to note that the present method of manufacturing the stabilized analog does not require extensive preprocessing such as grinding or milling of either the stabilizing agent or the drug. Also, heat is not required in order to melt the stabilizing agent or the drug, although heat may be applied in order to facilitate dissolution of the stabilizing agent into the solvent. This ability to form the stabilized camptothecin analog without melting or other preprocessing is a significant advantage of the method of the invention, as it reduces both manufacturing time and cost.

[00113] Although lyophilization is the preferred method for solvent removal, the solvent may also be removed by subjecting the mixture to rotary evaporation to yield a powder or a solid matrix. When a solid matrix is obtained, the material may be ground via ball milling or subjected to other mechanical shear stress to achieve a finely ground powder of nanoparticulate material. The resulting nanoparticles may be additionally stabilized with surfactants, phospholipids, stabilizing agents including albumin, and other stabilizing materials, as discussed above.

[00114] Another method of manufacturing the formulation is spray drying. In this method, a suitable organic solvent, ideally having a flash point sufficiently above the drying temperature, is used. Formulations made using this method are in the form of a fluffy, dry powder. Alternatively, the components of the final product may be dissolved in a supercritical fluid such as compressed carbon dioxide, and then ejected under pressure and shearing force to form dried particles of the drug-containing formulation.

[00115] The formulation is preferably stored in lyophilized form, in which case the lyophilized composition is rehydrated prior to use. Rehydration is carried out by mixing the lyophilized composition with an aqueous liquid (e.g., water, isotonic saline solution, phosphate buffer, etc.) to provide a total solute concentration in the range of about 10 to 100 mg/mL and a drug concentration in the range of about 0.02 to 20 mg/mL, preferably about 0.5 to 10 mg/mL. The formulation may, however, be stored in the aqueous state, e.g., in pre-filled syringes or vials. The formulation may also be stored as a liquid in a physiologically acceptable organic solvent such as ethanol, propylene glycol, or glycerol, to be diluted with water prior to injection into a patient. The lyophilized and rehydrated formulations may be stored at various temperatures, such as at freezing conditions (below about 0°C and as low as about -40°C to -100°C), refrigerated conditions generally between about 0°C and 15°C, room

temperature conditions generally between about 15°C and 2°C, or at elevated temperatures as high as about 40°C.

[00116] The particle size of individual particles within the formulation will vary, depending upon the molecular weight and concentration of the stabilizing agent, the amount of camptothecin analog as well as its solubility profile (i.e., its solubility in water and the hydrophilic polymer), the use of secondary stabilizing agents, and the conditions used in manufacturing. That is, as noted in the preceding section, secondary stabilizing agents and various excipients may be used to facilitate rehydration and provide a substantially homogeneous dispersion. Additionally, mechanical processing techniques can be used to adjust particle size to the appropriate diameter for the intended application; for example, after rehydration, the formulation can be subjected to shear forces with microfluidization, sonication, extrusion, or the like.

[00117] Formulations made with stabilizing agents can have a particle size on the order of about 20 nm to 100 nm. These smaller particles, by virtue of their larger accessible surface-to-volume ratio, tend to release drug quite rapidly, while larger particles, e.g., over 500 nm in diameter, will provide for far more gradual, sustained release of drug. The preferred particle size herein is in the range of about 1 nm to 1000 nm in diameter. For intramuscular and subcutaneous injection, the particle size should be in the range of about 1 nm to 500 nm, preferably in the range of about 10 nm to 30 nm, and most preferably in the range of about 20 nm to 200 nm. For intravenous administration, as noted previously, particle size is optimally in the range of about 200 nm to 800 nm. For interstitial administration and fracture or wound packing, particle size can be up to 1,000 nm, while for embolization, particle size will generally be between about 200 nm and 800 nm.

[00118] The formulation can be sterilized using heat, ionizing radiation, or filtration. For drugs that are thermally stable, heat sterilization is preferable. Lower viscosity formulations can be filter-sterilized, in which case the particle size should be under about 200 nm. Aseptic manufacturing conditions may be employed as well, and lyophilization is also helpful to maintain sterility and ensure a long shelf life. In addition, as noted in the preceding section, antibacterial agents may be included in aqueous formulations in order to prevent bacterial contamination.

[00119] Typical formulations of the invention are presented in Tables 1, 2, and 3 below. In Table 1 the drug is SN-38 and the stabilizers are saturated or unsaturated lipids. In Table 2

the drug is SN-38 and the stabilizers are a combination of lipid, poloxamine, and branched polyethylene glycols (bPEG) in various ratios. Table 3 illustrates various lipid components wherein at least one component is a PEGylated lipid.

TABLE 1

ID#	Component 1	Component 2	Composition Ratio*	Size of major peaks	24-hr stability
1	DOPG	-	1:15	67.1nm, 260.4nm	slight settling
2	DOPC	DOPG	1:5:10	65.4nm	slight settling
3	DOPC	DOPG	1:10:5	21.3nm	slight settling
4	DOPC	-	1:15	26.3nm	slight settling; phases separate
5	POPC	-	1:15	27.2nm	slight settling; phases separate
6	DLPC	-	1:17	194.9nm, 1050nm	slight settling; phases separate
7	DMPC	-	1:15	29.6nm	slight settling; phases separate
8	DPPC	-	1:15	2215nm	definite phase separation
9	DSPC	-	1:15	>3000nm	definite phase separation
10	POPC	-	1:30	20.3nm	slight settling; phases separate
11	POPC	-	1:15	26.4nm	slight settling; phases separate
12	POPC	-	2:15	37.0nm, 80.4nm	slight settling; phases separate
13	POPC	-	4:15	504.9nm, 2291nm	slight settling; phases separate
14	POPC	DOPG	1:18:2	22.1nm	transparent
15	POPC	DOPG	1:16:4	9.1nm	transparent
16	POPC	DOPG	1:19:1	34.2nm	transparent

*w/w ratio, wherein the first component is SN-38, and the subsequent components are the lipid component #1 and #2, respectively. In cases of settling or phase separation, the phases are easily resuspended.

TABLE 2

ID#	Component 1	Component 2	Composition Ratio*	Size of major peaks	24-hr stability
17	10k bPEG	DOPG	1:50:4	71.5nm, 395nm	definite phase separation
18	POPC	10k bPEG	1:20:8	25.6nm	definite phase separation
19	POPC	10k bPEG	1:20:4	24.9nm	definite phase separation

ID#	Component 1	Component 2	Composition Ratio*	Size of major peaks	24-hr stability
20	POPC	10k bPEG	1:20:2	25.0nm	definite phase separation
21	POPC	DOPG/10k bPEG	1:20:2:8	19.4nm	transparent
22	POPC	DOPG/ 10k bPEG	1:20:2:2	29.2nm	definite phase separation; translucent
23	Poloxamine	none	2:1	24.9nm	transparent
24	Poloxamine	DOPG	2:8:1	89.7nm, 297.1nm	transparent
25	Poloxamine	DOPG	2:20:1	191.6nm, 720.9nm	slight settling
26	Poloxamine	DOPG	2:40:1	170.7nm, 434.7nm	slight settling
27	POPC	Poloxamine	1:20:4	25.3nm	definite phase separation; translucent
28	POPC	Poloxamine	1:20:2	30.0nm	translucent
29	POPC	Poloxamine	1:20:1	25.7nm	definite phase separation; translucent
30	10k bPEG	DOPG/ Poloxamine	2:50:8:1	119.6nm, 373.9nm	slight settling

*w/w ratio, wherein the first component is SN-38, and the subsequent components are the lipid listed in the component #1 and #2, respectively. In cases of settling or phase separation, the phases are easily resuspended. Poloxamine is dialyzed before formulation. See Examples for details. 10k bPEG refers to 10,000 Dalton branched polyethylene glycol.

TABLE 3

ID#	Component 1	Component 2	Composition Ratio*	Size of major peaks	24-hr stability
31	POPC	POPE-PEG5000	1:12:8	25.3nm	slight settling
32	POPC	POPE-PEG5000	1:16:4	32.4nm	slight settling
33	POPC	POPE-PEG5000	1:18:2	30.9nm	slight settling
34	POPC	POPE-PEG5000	1:19:1	28.7nm	slight settling; phases separate no resuspension
35	POPC	POPE-PEG5000	2:39:1	33.8nm	slight settling; phases separate no resuspension
36	MRX-115*	none	1:15	30.7nm, 85.2nm	slight settling
37	MRX-115	none	1:30	58.9nm	slight settling
38	MRX-115	none	1:5	20.3nm, 73.3nm	slight settling

ID#	Component 1	Component 2	Composition Ratio*	Size of major peaks	24-hr stability
39	MRX-115u	none	1:5	100.6nm, 389.2nm	slight settling
40	MRX-115u	none	1:10	54.8nm	slight settling
41	MRX-115u	none	1:20	33.2nm	slight settling; phases separate

MRX-115 is a mixture of lipids comprised of DPPC:DPPA:DPPE-PEG5000 in the molar ratio of 80:10:15. MRX-115u designates a mixture of DOPC:DOPA:DOPE-PEG5000, the corresponding unsaturated lipids in the same molar ratio. Except where noted otherwise, all separated phases are readily resuspended.

D. INCORPORATION OF AN ACOUSTICALLY ACTIVE GAS

[00120] In a further embodiment of the invention, the present formulations are made with small quantities of an acoustically active gas instilled therein. In order to instill the selected gas into the present formulations, a headspace of gas (preferably an insoluble gas) is applied atop the lyophilized composition in a closed container, which is then exposed to mild agitation during rehydration. Small quantities of gas will become entrapped in the interstices of the dispersion. The presence of the acoustically active gas is useful in conjunction with ultrasound imaging, as the gas-instilled dispersion produces an echogenic contrast that allows the drug to be tracked in the body. In addition, if a sufficient quantity of gas is entrapped in the formulation, therapeutic ultrasound can allow the microstructure to unfold at the locus where the ultrasound is applied, releasing the camptothecin analog and thus enhancing targeting effectiveness. The acoustically active gas lowers the cavitation threshold, i.e., the energy required for cavitation with ultrasound. Preferably, the cavitation energy used will be under about 1.5 MPa, and more preferably under about 1.0 MPa. The gas also effects dB reflectivity, and a gas concentration of about 1 mg per mL of particles will generally have a reflectivity approximately 2 dB higher than that of pure water.

[00121] In general, the amount of acoustically active gas that is imbibed by the particles of the formulation is approximately equal to the void space within the particles, which can be approximated by their density. For example, particles having a density of 0.10 will imbibe about 90 vol% gas. Lower density particles will imbibe a higher volume of gas (e.g., 95 vol% for particles having a density of 0.05), while higher density particles will imbibe a lower volume of gas (e.g., 85 vol% for particles having a density of 0.15). Gas may also adhere to the surface of the particles, typically up to about two times the volume of the particles.

Normally, the amount of acoustically active gas that is employed is such that the gas-instilled formulation will contain at least about 5 vol% gas, preferably about 10-15 vol% gas.

[00122] Typical acoustically active gases are chemically inert gases having 1 to 12 carbon atoms, and particularly preferred acoustically active gases are perfluorocarbons, including saturated perfluorocarbons, unsaturated perfluorocarbons, and cyclic perfluorocarbons. The saturated perfluorocarbons, which are usually preferred, have the formula C_nF_{2n+2}, where n is from 1 to 12, preferably 2 to 10, more preferably 4 to 8, and most preferably 5. Examples of suitable saturated perfluorocarbons are the following: tetrafluoromethane; hexafluoroethane; octafluoropropane; decafluorobutane; dodecafluoropentane; perfluorohexane; and perfluoroheptane. Saturated cyclic perfluorocarbons, which have the formula C_nF_{2n}, where n is from 3 to 8, preferably 3 to 6, may also be preferred, and include, e.g., hexafluorocyclopropane, octafluorocyclobutane, and decafluorocyclopentane. Other gases that can be used include air, nitrogen, helium, argon, xenon, and other such gases.

[00123] Alternatively, a gaseous precursor can be used that is in the liquid state at room temperature and that is either (1) volatilized prior to introduction into the headspace above the lipid- and drug-containing dispersion, or (2) volatilized and instilled into a microemulsion that is then introduced into the lipid- and drug-containing dispersion. Suitable gaseous precursors are described, for example, in U.S. Patent No. 5,922,304 to Unger, and include, without limitation: hexafluoro acetone, isopropyl acetylene, allene, tetrafluoroallene, boron trifluoride, isobutane, 1,2-butadiene, 2,3-butadiene, 1,3-butadiene, 1,2,3-trichloro-2-fluoro-1,3-butadiene, 2-methyl-1,3-butadiene, hexafluoro-1,3-butadiene, butadiyne, 1-fluoro-butane, 2-methyl-butane, decafluorobutane, 1-butene, 2-butene, 2-methyl-1-butene, 3-methyl-1-butene, perfluoro-1-butene, perfluoro-2-butene, 4-phenyl-3-butene-2-one, 2-methyl-1-butene-3-yne, butyl nitrate, 1-butyne, 2-butyne, 2-chloro-1,1,1,4,4,4-hexafluorobutyne, 3-methyl-1-butyne, perfluoro-2-butyne, 2-bromobutyraldehyde, carbonyl sulfide, crotononitrile, cyclobutane, methyl-cyclobutane, octafluorocyclobutane, perfluorocyclobutene, 3-chlorocyclopentene, octafluorocyclopentenecyclopropane, 1,2-dimethyl-cyclopropane, 1,1-dimethylcyclopropane, 1,2-dimethylcyclopropane, ethylcyclopropane, methylcyclopropane, diacetylene, 3-ethyl-3-methyl diaziridine, 1,1,1-trifluorodiazoethane, dimethyl amine, hexafluorodimethylamine, dimethylethylamine, bis(dimethylphosphine)amine, perfluorohexane, 2,3-dimethyl-2-norbornane, perfluorodimethylamine, dimethyloxonium chloride, 1,3-dioxolane-2-one, 4-methyl-1,1,1,2-tetrafluoroethane, 1,1,1-trifluoroethane,

1,1,2,2-tetrafluoroethane, 1,1,2-trichloro-1,2,2-trifluoro-ethane, 1,1-dichloroethane, 1,1-dichloro-1,2,2,2-tetrafluoroethane, 1,2-difluoroethane, 1-chloro-1,1,2,2,2-pentafluoroethane, 2-chloro-1,1-difluoroethane, 1,1-dichloro-2-fluoroethane, 1-chloro-1,1,2,2-tetrafluoroethane, 2-chloro-1,1-difluoroethane, chloroethane, chloropenta-fluoroethane, dichlorotrifluoroethane, fluoroethane, hexafluoroethane, nitropentafluoroethane, nitrosopentafluoroethane, perfluoroethylamine, ethyl vinyl ether, 1,1-dichloroethane, 1,1-dichloro-1,2-difluoroethane, 1,2-difluoroethane, methane, trifluoromethanesulfonylchloride, trifluoromethane-sulfonylfluoride, bromodifluoronitrosomethane, bromofluoromethane, bromochloro-fluoromethane, bromotrifluoromethane, chlorodifluoronitromethane, chlorodinitromethane, chlorofluoromethane, chlorotrifluoromethane, chlorodifluoromethane, dibromodifluoromethane, dichlorodifluoromethane, dichlorofluoromethane, difluoromethane, difluoroiodo-methane, disilanomethane, fluoromethane, iodomethane, iodotrifluoromethane, nitrotrifluoromethane, nitrosotrifluoromethane, tetrafluoromethane, trichlorofluoromethane, trifluoromethane, 2-methylbutane, methyl ether, methyl isopropyl ether, methyllactate, methylnitrite, methylsulfide, methyl vinyl ether, neon, neopentane, nitrogen (N_2), nitrous oxide, 1,2,3-nonadecane-tricarboxylic acid-2-hydroxytrimethylester, 1-nonene-3-yne, oxygen (O_2), 1,4-pentadiene, n-pentane, perfluoropentane, 4-amino-4-methylpentan-2-one, 1-pentene, 2-pentene (cis), 2-pentene (trans), 3-bromopent-1-ene, perfluoropent-1-ene, tetrachlorophthalic acid, 2,3,6-trimethylpiperidine, propane, 1,1,1,2,2,3-hexafluoropropane, 1,2-epoxypropane, 2,2-difluoropropane, 2-aminopropane, 2-chloropropane, heptafluoro-1-nitropropane, heptafluoro-1-nitrosopropane, perfluoropropane, propene, hexafluoropropane, 1,1,1,2,3,3-hexa-fluoro-2,3 dichloropropane, 1-chloropropane, chloropropane (trans), 2-chloropropane, 3-fluoropropane, propyne, 3,3,3-trifluoropropyne, 3-fluorostyrene, sulfur hexafluoride, sulfur (di)-decafluoride(S_2F_{10}), 2,4-diaminotoluene, trifluoroacetonitrile, trifluoromethyl peroxide, trifluoromethyl sulfide, tungsten hexafluoride, vinyl acetylene, vinyl ether, and xenon.

E. UTILITY

[0100] The formulations of the invention are used to treat a mammalian individual, generally a human patient, suffering from a condition, disease, or disorder that is responsive to systemic administration of a camptothecin derivative. The formulations may be administered orally, parenterally, topically, transdermally, rectally, vaginally, by inhalation,

intraocularly, intranasal, sublingually, in an implanted reservoir (i.e., in a sustained release depot for subcutaneous or intramuscular administration), or as a packing material for wounds and fractures. The term "parenteral" as used herein is intended to include subcutaneous, intravenous, intramuscular, intra-arterial, intrathecal, and intraperitoneal injection, and the formulation may be injected as either a bolus or an infusion. Therefore, one embodiment of the invention is a method for delivering a drug to a mammalian individual to achieve a desired therapeutic effect, comprising administering to the individual a therapeutically effective amount of a formulation of the invention, e.g., intravenously, orally, parenterally, intraperitoneally, subcutaneously or via injection into a body cavity such as a joint, or via inhalation for delivery to the lungs.

[0101] In another embodiment of the invention the method involves treating an individual suffering from cancer, and comprises parenterally administering to the patient a spatially stabilized matrix formulation of: (a) drug-containing particles comprised of (i) a stabilizing agent, (ii) a camptothecin analog that complexes with but does not covalently bind to the stabilizing agent, optionally (iii) a targeting ligand, and optionally (iv) an excipient selected from the group consisting of saccharides, liquid polyethylene glycols, propylene glycol, glycerol, ethyl alcohol, and combinations thereof, in (b) an aqueous vehicle.

[0102] Table 4 shows comparative data from production of nanoparticles of camptothecin and SN-38. Several surprising and unexpected findings are evident. Firstly, nanoparticles prepared with camptothecin are much larger than nanoparticles prepared with SN-38. Secondly, nanoparticles prepared with the addition of an anionic lipid (phosphatidylglycerol) are much smaller than the particles prepared without the anionic lipid. A neutral lipid, by comparison, prepares much larger particles, which settle quickly. The particles prepared with phosphatidylglycerol remain in solution as a homogeneous suspension.

TABLE 4

ID #	COMPONENTS	RATIO	POST FLUIDIZATION	24 HOUR STABILITY
42	POPG: 123.8mg Poloxamer 188: 15.0mg SN-38: 30mg 60ml 0.50mg/ml Stock Solution	SN-38 : POPG : Poloxamer 2:8:1	Intensity Weighted: 105.0 nm 30.7% 286.4 nm 69.3% Volume Weighted: 102.2 nm 67.1% 289.2 nm 32.9% Number Weighted: 98.8 nm 98.0% 285.7 nm 2.0%	Trace amount white and brownish sediment after 24 hrs. No phase separation, stable.
43	POPC: 124.2mg Poloxamer 188: 15.0mg SN-38: 30mg 60ml 0.50mg/ml Stock Solution	SN-38 : POPC : Poloxamer 2:8:1	Intensity Weighted: 268.5 nm 26.9% 942.3 nm 73.1% Volume Weighted: 272.5 nm 6.7% 962.6 nm 93.3% Number Weighted: 266.9 nm 76.4% 952.6 nm 23.6%	Suspension settles loosely to bottom of vial leaving a clear supernatant after 24 hrs. Re-suspends easily.
44	Poloxamer 188: 15.0mg SN-38: 30mg 60ml 0.50mg/ml Stock Solution	SN-38 : Poloxamer 2:1	Intensity Weighted: 234.8 nm 99.6% Volume Weighted: 235.7 nm 95.0% 1359.9 nm 5.0% Number Weighted: 197.5 nm 100.0%	Trace amount white sediment after 24 hrs. No phase separation, stable.
45	POPG: 121.2mg Poloxamer 188: 15.0mg Camptothecin: 30mg 50ml 0.63mg/ml Stock Solution	CPT : POPG : Poloxamer 2:8:1	Intensity Weighted: 254.8 nm 82.9% 1859.1 nm 17.1% Volume Weighted: 257.6 nm 35.0% 1783.1 nm 65.0% Number Weighted: 251.5 nm 99.4%	No sedimentation, stable for less than 24 hrs.
46	POPC: 121.8mg Poloxamer 188: 15.0mg Camptothecin: 30mg 50ml 0.63mg/ml Stock Solution	CPT : POPC : Poloxamer 2:8:1	Intensity Weighted: 256.5 nm 28.0% 989.8 nm 72.0% Volume Weighted: 259.5 nm 9.6% 1002.9 nm 90.4% Number Weighted: 254.4 nm 86.0% 984.9 nm 14.0%	Suspension settles loosely to bottom of vial leaving a supernatant that is more transparent than the original suspension, less than 24 hrs. Re-suspends easily.
47	Poloxamer 188: 15.0mg Camptothecin: 30mg 50ml 0.63mg/ml Stock Solution	CPT : Poloxamer 2:1	Intensity Weighted: 309.9 nm 100.0% Volume Weighted: 400.0 nm 100.0% Number Weighted: 233.8 nm 100.0%	No sedimentation, stable for less than 24 hrs.

[0103] The levels of anticancer efficacy for the formulations of the invention represent a significantly improved profile when compared with CAMPTOSAR®, a water-soluble, FDA-

approved form of camptothecin. Details of the efficacy studies and an interpretation of the results are presented in Examples 11 and 12.

[0104] The present formulations are also useful as packing materials for wounds and fractures, and as coating materials for endoprostheses such as stents, grafts, and joint prostheses. It is known that restenosis (narrowing of the blood vessels) may occur after angioplasty, placement of a stent, and/or other coronary intervention procedures, as a result of fibroblast proliferation and smooth muscle hypertrophy. Thus, the formulations of the invention may be used as coating materials for endoprostheses to provide local drug delivery following coronary intervention to, for example, prevent or inhibit restenosis.

[0105] It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, the foregoing description as well as the examples that follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages, and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

EXPERIMENTAL

[0106] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to prepare and use the formulations disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperatures, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in degrees Celsius (°C), and pressure is at or near atmospheric.

[0107] All materials were purchased from commercial sources such as Polymer Source (Dorval, Canada), Avanti Polar Lipids (Alabaster, AL), Genzyme Pharmaceuticals (Cambridge, MA), or Northern Lipids (Vancouver, British Columbia). All other materials were obtained as follows: SN-38 (from Decode Genetics, Woodbridge, IL); paclitaxel (Natural Pharmaceuticals, Inc., Beverly, MA); poloxamer (Poloxamer 188, BASF, Parsippany, NJ); mannitol (Aldrich Chemical Company, Milwaukee, WI); and sorbitol (Fischer Chemical, Fairlawn, NJ).

EXAMPLE 1

PREPARATION OF FORMULATION 24 IN TABLE 2:

PROCEDURE FOR MAKING POLOXAMINE-STABILIZED COMPOSITION:

[0108] Twenty-five milliliters of SN-38 formulation were made (composition ratio 2:8:1 SN-38:DOPG:poloxamine, 1 mg/mL SN-38, 4 mg/mL DOPG, 0.5 mg/mL poloxamine) using a small microfluidizer.

[0109] 120 mg of DOPG was dissolved with 40 mL of t-butanol in a 250 mL round-bottom flask by heating for a few minutes on a Rotovap. SN-38 stock solution, 0.5 mg/mL in dichloromethane/tert-butanol (1:1), was added to the DOPG solution in the flask until the desired SN-38 concentration was reached. (60 mL of stock solution was used to achieve a 1.0 mg/mL SN-38 concentration.) The flask was placed on a Rotovap, and heated for 15 min to remove the dichloromethane. The flask was flash-frozen with liquid nitrogen and freeze-dried overnight.

[0110] The formulation was rehydrated with 25 mL of un-buffered poloxamine solution (0.5 g poloxamine, diluted to 1 L with purified water) and allowed to sit for 30-60 min, shaking occasionally, until no large clumps of material were present. A microfluidizer was rinsed with the rehydration solution to fill 5 mL of microfluidizer dead volume and to achieve 30 mL final formulation rehydration volume. The solution was microfluidized for 20 min at a pressure of approximately 50 psig. The resulting suspension was faintly yellow and translucent with some birefringence. Some settling of particulate matter occurred after 72 hrs refrigeration.

EXAMPLE 2

PREPARATION OF FORMULATION 33 IN TABLE 3:

PROCEDURE FOR MAKING 25 ML OF SN-38 FORMULATION (COMPOSITION RATIO 1:18:2 SN-38:POPC:DOPG, 1MG/ML SN-38, 18MG/ML POPC, 2MG/ML DOPG)

[0111] 540.0 mg of POPC and 60.0 mg DOPG were dissolved in 40 mL of t-butanol in a 250 mL round-bottom flask by heating for a few minutes on a Rotovap. Note that heating was only used to speed dissolution. To the POPC/DOPG solution was added 0.5 mg/mL SN-38 stock solution until the desired SN-38 concentration was reached. (60 mL of stock solution was used to achieve a 1.0 mg/mL SN-38 concentration.) The flask was placed on a

Rotovap, and heated for 15 min to remove the dichloromethane. The flask was the flash-frozen with liquid nitrogen and freeze-dried overnight.

[0112] The freeze-dried formulation was then rehydrated with a 0.001 M citrate buffer, pH 5. The citrate buffer solution was made combining 5.9 mL 0.1 M sodium citrate and 4.1 mL 0.1 M citric acid and diluting to 1 L with purified water. The pH was adjusted to 5 ± 0.1. The hydrated formulation was allowed to sit for 20-30 min, shaking occasionally. The formulation was then sonicated for 10-20 min until no large clumps of material were present. A microfluidizer was rinsed with rehydration solution to fill 5 mL of microfluidizer dead volume and achieve 30 mL final formulation rehydration volume. The solution was then microfluidized for 20 min at a pressure of approximately 50 psig. 25 mL of formulation was collected from the microfluidizer via syringe. The resulting suspension was pale-yellow, translucent, non-birefringent, and stable under prolonged refrigeration.

EXAMPLE 3

PREPARATION OF FORMULATION 41 IN TABLE 3:

UNSATURATED STABILIZED LIPID BLEND FORMULATION:

[0113] Procedure for making 25 mL of SN-38 (formulation #41 from Table 3) (composition ratio 1:20 SN-38: unsaturated blend, 1mg/mL SN-38, 20mg/mL mixture of lipid blend containing POPC:POPA:POPE-PEG 5000 in the weight ratio of 54:6:40.) using a small microfluidizer.

[0114] 600.0 mg of an unsaturated blend (blend comprised of 324.0 mg POPC, 240 mg POPE-PEG 5000, and 36 mg POPA) was dissolved in 40 mL of t-butanol in a 250 mL round-bottom flask by heating for a few minutes on a Rotovap. SN-38 stock solution, 0.5 mg/mL, was added to the unsaturated lipid blend solution in the flask until the desired SN-38 concentration was reached. (60 mL of stock solution was used to achieve a 1.0 mg/mL SN-38 concentration.) The flask was placed on a Rotovap, and heated for 15 min to remove the dichloromethane. The flask was then flash-frozen with liquid nitrogen and freeze-dried overnight.

[0115] The formulation was then rehydrated with 25 mL of 0.001 M citrate buffer, pH 5. The citrate buffer solution was made combining 5.9 mL 0.1 M sodium citrate and 4.1 mL 0.1 M citric acid and diluting to 1 L with purified water. The pH was adjusted to 5 ± 0.1. The hydrated formulation was allowed to sit for 20-30 min, with occasional shaking. The

formulation was then sonicated for 20-30 min until no large clumps of material were present. A microfluidizer was then rinsed with rehydration solution to fill 5 mL of microfluidizer dead volume and achieve 30 mL final formulation rehydration volume. The solution was then microfluidized for 20 min at a pressure of approximately 50 psi. 25 mL of the formulation was collected from the microfluidizer via syringe.

[0116] The resulting suspension was pale-yellow, transparent, non-birefringent, and settled only slightly after 24 hrs under prolonged refrigeration.

EXAMPLE 4

PREPARATION OF FORMULATION 42 IN TABLE 4

[0117] The procedure described in Example 1 was duplicated, substituting an equivalent amount of poloxamer for poloxamine.

EXAMPLE 5

PREPARATION OF FORMULATION 43 IN TABLE 4

[0118] The procedure was identical to that in Example 4, but substituted POPC for POPG.

EXAMPLE 6

PREPARATION OF FORMULATION 44 IN TABLE 4

[0119] The procedure from Example 1 was followed for the initial solubilization of SN-38 to the same concentration. The same flash-freezing and freeze-drying procedures were followed. In this instance, however, the freeze-dried formulation was rehydrated with 25 mL of an un-buffered poloxamine solution (0.5 g poloxamine, diluted to 1 L with purified water).

[0120] The hydrated formulation was allowed to sit for 30-60 min, shaking occasionally, until no large clumps of material were present. A microfluidizer was rinsed with rehydration solution to fill 5 mL of microfluidizer dead volume and achieve 30 mL final formulation rehydration volume and the solution was microfluidized for 20 min at a pressure of approximately 50 psi. 25 mL of the formulation was collected from the microfluidizer via syringe.

EXAMPLE 7

PREPARATION OF FORMULATION 45 IN TABLE 4

[0121] The procedure was identical to that followed in Example 4 except for the substitution of camptothecin for SN-38.

EXAMPLE 8

PREPARATION OF FORMULATION 46 IN TABLE 4

[0122] The procedure was identical to that followed in Example 5 except for the substitution of camptothecin for SN-38.

EXAMPLE 9

PREPARATION OF FORMULATION 47 IN TABLE 4

[0123] The procedure was identical to that followed in Example 6 except for the substitution of camptothecin for SN-38.

EXAMPLE 10

LYOPHILIZATION PROCEDURE FOR FORMULATION 23

[0124] A formulation containing SN-38, poloxamine, and sucrose was lyophilized in the following manner:

[0125] The SN-38/poloxamine formulation was prepared using the standard method of lyophilization from t-butanol, and rehydrated in purified water. The rehydrated formulation was microfluidized, and sucrose was added after the fluidization step. 1 mL aliquots of the formulation were transferred to 2 cc, 13 mm flint glass tubing vials (Helvoet, Pennsauken, New Jersey). The vials were stoppered with 13 mm lyo- type rubber stoppers (Daikyo-Seiko, Japan) in the lyo-position and placed in a Unitop SQ Drying Stoppering chamber equipped with a Freezemobile research-scale freeze-dryer (Virtis Company, Gardiner, New York). The formulation was then lyophilized using a standard 2-step lyophilization cycle. The resulting product was a uniform yellowish cake that rehydrated readily with gentle shaking.

[0126] For formulations containing both phospholipids and poloxamine or poloxamer, the lipids and camptothecin drugs were co-formulated prior to lyophilization and the poloxamine/poloxamer was added during rehydration.

EXAMPLE 11

ALTERNATIVE FORMULATION METHOD

[0127] A formulation containing SN-38, polaxamine, and sucrose was processed in the following manner.

[0128] The lyophilization steps from Example 10 were followed, except the lyophilisate was resuspended in 0.001 M citrate buffer, pH 5. The citrate buffer solution was made combining 5.9 mL 0.1 M sodium citrate and 4.1 mL 0.1 M citric acid and diluting to 1 L with purified water. The pH was adjusted to 5 ± 0.1. Half of the rehydrated formulation was then microfluidized for 20 min. The other aliquot was extruded using an SP extruder (SP Pharmaceuticals, Albuquerque, New Mexico) fitted with a series of 200nm, 80nm, 50nm and 90nm polycarbonate filters (Whatman, Kent, UK). The fluidized sample was then extruded the same way and particle sizes were compared. Both formulations showed a volume-weighted size of less than 300nm.

EXAMPLE 12

ANTI-TUMOR EFFICACY OF SN-38 FORMULATIONS

[0129] A culture of HT-29 human colon adenocarcinoma cells from ATCC was grown in McCoy's 5a medium with L-glutamine, sodium bicarbonate, and 10% fetal calf serum at 37°C under an atmosphere of 5% CO_2. Cells were collected with trypsin-EDTA and spun at 250 x g. A final dilution was prepared at 5 million cells per milliliter.

[0130] Two 100-microliter injections of cells were given to nude mice to form tumors in the upper leg region. At seven days following inoculation, the mice had treatments initiated with 500 microliter inocula of each formulation. Control mice were untreated. For Camptosar®, the concentrations of active compound were adjusted to be approximately 6X higher than for the amounts of SN-38 in experimental animals. Also, Camptosar was administered daily while the SN-38 treated animals were dosed twice weekly. After 14 days of treatment, no further inocula of Camptosar or SN-38 formulations were administered in order to assess the duration of efficacy. The experiments were terminated when tumor growth reached 1 gram.

[0131] Results of the study comparing untreated (control), Camptosar-treated and SN-38 formulations are shown in Figure 1. JDW98B and JDW98D are formulations of SN-38 with branched polyethylene glycol, wherein 98B is comprised of SN-38:10kD bPEG:DOPG at a

ratio of 1:50:4 and JDW98D is comprised of SN-38:10kD bPEG:DOPG at a ratio of 1:25:2. It is evident that after an initial lag time of 1-2 weeks SN-38-containing formulations are more effective at inhibiting the growth of tumor masses than Camptosar. Also notable is the duration of the efficacy. Tumors in mice treated with Camptosar begin to increase in weight within 24 hrs after the last inoculation. The resumption of tumor growth in animals treated with SN-38:branched PEG formulations does not begin for 10 days after the last dose, indicating a sustained release profile in circulation. Toxicity data (not shown) as measured by total weight gain/loss of animals during the treatment intervals shows that SN-38:branched PEG is well tolerated. Animals returned to full initial weights after cessation of treatment.

EXAMPLE 13

COMPARATIVE EFFICACIES OF VARIOUS LIPIDIC STABILIZED SN-38 FORMULATIONS

[0132] Another set of experiments was conducted, run identically to those described in Example 12 but with all test formulations containing SN-38. Formulation 118A corresponds to #23 from Table 2 above, containing no lipidic stabilizers. Formulation 138A corresponds to #36 from Table 3; formulation 140°C corresponds to #2 from Table 1, and formulation 142B corresponds to #39 from Table 3. All animals were tested in groups of four mice (eight tumors). It is apparent from Figure 2 that all lipidic and non-lipidic stabilized formulations of SN-38 exhibit comparable and statistically indistinguishable antitumor efficacy, which in all cases is positive.

EXAMPLE 14

COMPARATIVE SERUM STABILITY OF CAMPTOTHECIN AND SN-38

[0133] A quasi-serum is made by dissolving HSA (human serum albumin) in water to a concentration of 20 mg/mL. The solution is heated to 37°C, and aliquots of 50 microliters of camptothecin or SN-38 formulations are added to produce a final dilution of 1:40. Kinetics of lactone ring opening are monitored by HPLC. Testing over 50 samples containing unstabilized camptothecin and a like number of stabilized SN-38, it was found that the average equilibrium percentage of lactone ring form was approximately 10% for camptothecin and over 60% for SN-38. The range for camptothecin samples was from 3-26% and for SN-38 from 50-94%. Clearly, the lactone ring is more stable to opening and subsequently less susceptible to loss of bioactivity in all SN-38 formulations compared to

camptothecin formulations. Thus, it can be concluded that the chemical stability is greater and the therapeutic window for SN-38 is significantly enhanced in contrast to camptothecin.

EXAMPLE 15

FORMULATION OF SN-38 BY SUPERCRITICAL FLUID TECHNOLOGY

[0134] Each of the formulations enumerated in Tables 1-4 can be produced by alternative methodologies to those described in the Examples above. One preferred formulation methodology utilizes supercritical fluid solubilization followed by extrusion through a nozzle. In this method, the lipidic suspension of SN-38 and lipids as described in Example 1 is stirred into liquid carbon dioxide. Following this procedure, the material is collected and microfluidized, with subsequent procedures used as described above. As one skilled in the art would recognize supercritical fluid processing may also be performed with other solvents and cosolvents besides carbon dioxide, depending upon the solubility properties of the drug and the stabilizing materials.

EXAMPLE 16

FORMULATION OF 7-ETHYLCAMPTOTHECIN NANOPARTICLES

[0135] An unsaturated lipid blend formulation is made using 7-ethylcamptothecin by substituting 7-ethylcamptothecin for SN-38 and using the method described in Example 1.

EXAMPLE 17

ELECTRON MICROSCOPY OF SN-38 FORMULATIONS

[0136] Cryo-electron microscopy was performed on frozen hydrated specimens. In this example, formulation #37 from table 3 was used. A thin layer (200-400 nm) of a suspension of the nanoparticles was created by applying 4-microliter droplets of the suspension to the coated surface of "lacey" carbon-coated EM grids and blotting away the excess liquid with a piece of filter paper from the back side of the grids. Plunging the grids into liquid propane cooled to near liquid nitrogen temperature vitrified the thin layer of suspended nanoparticles. The specimens were stored in special grid holders in liquid nitrogen until cryotransfer and observation at -170°C on a GATAN model 626 TEM cold stage, in a Philips 420 TEM.

EXAMPLE 18

ALTERNATIVE FORMULATION METHOD

[0137] SN-38 (5mg/mL) and sorbitol (100 mg/mL) were dissolved in warm DMSO. 6.75 mL of this solution was combined with a mixture of 900 mg POPC, 45 mg DOPG, 22.5 mg DOPE-PEG 5000, and 450 mg PEG 600 in 128.25 mL of tert-butyl alcohol (t-butanol). The solution was filtered with a sterilizing filter and aliquotted into vials. The vials were placed in a lyophilizer and lyophilized according to a standard cycle. The resulting powder was rehydrated with purified water and sonicated for 60 seconds. The particle size of the resulting suspension was approximately 300 nm.

EXAMPLE 19

ALTERNATIVE FORMULATION METHOD

[0138] SN-38 (2g) and sorbitol (15g) were dissolved in 150 mL of DMSO. 20g of POPC, 1g DOPG, 0.5g DOPE-PEG 5000, and 10g 6K linear PEG were dissolved in 850 mL of *t*-butanol in a separate beaker. The solutions were heated to no more than 75°C to dissolve the components. The DMSO and the *t*-butanol solutions were combined, and stirred to mix. The resulting solution was sterile-filtered through a 0.2 μm nylon filter, and 9-mL aliquots were filled into 20cc vials. The vials were stoppered in the lyo-position and lyophilized using a standard cycle.

concentrations	mg/mL	molar	ratio to SN-38
SN-38	1.5	0.004	NA
POPC	5	0.007	1.72 : 1
DOPG	1	0.001	0.33 : 1
POPE-PEG2K	20	0.007	1.88 : 1
Sorbitol	5	0.027	7.18 : 1

EXAMPLE 20

ALTERNATIVE FORMULATION METHOD

[0139] A solution of four-arm poly(ethylene oxide-b-lactide) L form (5 mg/mL) in t-butanol was combined with SN-38 (0.5 or 1 mg/mL), DOPG (0.28 or 0.56 mg/mL), and DOPE-PEG 5000 (0 or 0.25 mg/mL). The samples were flash-frozen, and lyophilized. The

resulting powders were rehydrated with a 1mM citrate buffer and sonicated. The particle size of the resulting solutions showed a particle size range of 250nm to 3500 nm.

EXAMPLE 21

ALTERNATIVE FORMULATION METHOD

[0140] All the ingredients were weighed out into a round bottom flask. The ingredients were dissolved in 5% DMSO/95% t-butanol. In order to solubilize the excipients, the fill volume used in each vial was 3 times the required hydration volume. The solution was filtered through a 0.2 μm DMSO-safe filter directly into sterile vials. The vials were frozen at -50°C for 2 hrs, followed by a lyophilization cycle at -5°C for 16 hrs and a secondary drying cycle at 40°C for 6 hrs. The vials containing dry powder were then sealed under a partial vacuum.

[0141] Alternatively, all the excipients were dissolved in 95 % tert-butanol, optionally heating to 75°C and SN-38 was dissolved in 5% DMSO separately. The dissolved SN-38 was then added directly to the solution of excipients in tert-butanol and aliquoted into vials.

[0142] The following are some of the formulation compositions that have been prepared using these methods:

a) 10 mg/mL POPC;
0.5 mg/mL DOPE-PEG 5K;
25 mg/mL 6K linear PEG; and
1 mg/mL SN-38.

b) 10 mg/mL POPC;
0.5 mg/mL DOPE-PEG 5K;
25 mg/mL 6K linear PEG;
2.5 mg/mL Poloxamer; and
1 mg/mL SN-38.

c) 20 mg/mL POPC;
1 mg/mL DOPG;
25 mg/mL 10K linear PEG; and
1 mg/mL SN-38.

d) 2 mg/mL four-arm poly (ethylene oxide-b-caprolactone) (branched PEG-b-polycaprolactone);
0.5 mg/mL DOPE-PEG 5K;
20 mg/mL POPC;
15 mg/mL 6K linear PEG; and
0.75 mg/mL SN-38.

e) 5 mg/mL branched PEG-b-polycaprolactone;
1 mg/mL DOPG; and
1 mg/mL SN-38.

f) 10 mg/mL DOPE-PEG 2k;
1 mg/mL DOPG;
5 mg/mL branched PEG-b-polycaprolactone;
5 mg/mL sorbitol; and
1.5 mg/mL SN-38.

g) 20 mg/mL sorbitol;
1 mg/mL DOPG;
5 mg/mL branched PEG-b-polycaprolactone; and
1.5 mg/mL SN-38.

EXAMPLE 22

[0143] The SN-38, branched PEG-b-polycaprolactone, and DOPG were dissolved in an amount of DMSO equal to 5% of the final volume. A 1.2% (w/v) mannitol solution was prepared in an amount of water equal to 25% of the final volume. The mannitol solution was combined with an amount of tert-butyl alcohol equal to 70% of the final volume. The DMSO solution was combined with the mannitol/water/TBA solution and mixed thoroughly. The resulting clear solution was sterile filtered through a 0.2μm filter and filled into 10cc vials at a fill volume of 4.5 mL. The vials were loaded unto -45°C shelves and lyophilized according to a standard cycle. The resulting lyophilized cake was easily hydrated with water for injection to yield a translucent solution which contained very little crystalline matter when observed under a light microscope. Electron microscopy showed that the particles had a rod-like structure that was 1 to 5μm in length.

[0144] Optimal Ratios for the formulation:

Concentrations*	mg/mL	molar	ratio to SN-38
SN-38	1.5	0.004	NA
Caprolactone	10	0.001	0.22 : 1
DOPG	1	0.001	0.33 : 1
Mannitol	20	0.329	86.1 : 1

CLAIMS

1. A pharmaceutical formulation comprising:

a camptothecin analog;

a stabilizing agent that stabilizes the camptothecin analog but does not covalently bind thereto;

an optional targeting ligand; and

an optional excipient.

2. The formulation of claim 1, wherein the stabilizing agent comprises a polymer, a lipid, a polymer-lipid conjugate, or a combination thereof.

3. The formulation of claim 2, wherein the stabilizing agent is a polymer.

4. The formulation of claim 3, wherein the polymer is selected from linear and branched structures.

5. The formulation of claim 4, wherein the polymer is a block copolymer.

6. The formulation of claim 5, wherein the polymer is a branched block copolymer selected from polyethylene glycol-polypropylene oxide, polyethylene glycol-polylactide, polyethylene glycol-polylactide-coglycolide, and polyethylene glycol-b-polycaprolactone copolymers.

7. The formulation of claim 5, wherein the polymer is a branched block copolymer having a central core and about 3 to 12 arms radiating therefrom.

8. The formulation of claim 7, wherein each arms comprises a block copolymer with an inner, more hydrophobic block and an outer, more hydrophilic block.

9. The formulation of claim 7, wherein each arms comprises a block copolymer with an inner, more hydrophilic block and an outer, more hydrophobic block.

10. The formulation of claim 3, wherein the polymer is selected from polyethylene glycol, polyglycolide, polyvinyl alcohol, polyvinyl pyrrolidone, polylactide, poly(lactide-co-glycolide), polycaprolactone, polysorbate, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide), poloxamer, poloxamine, poly(oxyethylated) glycerol, poly(oxyethylated) sorbitol, poly(oxyethylated) glucose, and derivatives, mixtures, and copolymers thereof.

11. The formulation of claim 3, wherein the polymer is poloxamer.

12. The formulation of claim 3, wherein the polymer is poloxamine.

13. The formulation of claim 3, wherein the polymer is selected from the group consisting of a polyethylene glycol and polypropylene glycol and copolymers thereof.

14. The formulation of claim 13, wherein the polymer is selected from branched polyethylene glycol, linear polyethylene glycol, and combinations thereof, and is optionally covalently bound to at least one phospholipid moiety.

15. The formulation of claim 13, wherein the polyethylene glycol is functionalized to contain at least one sulfhydryl, amino, lower alkoxy, carboxylate, or phosphonate moiety.

16. The formulation of claim 13, wherein the polyethylene glycol or polypropylene glycol contains a hydrolyzable linkage.

17. The formulation of claim 13, wherein the polyethylene glycol is bonded to a phospholipid moiety.

18. The formulation of claim 17, wherein the polyethylene glycol ranges in size from about 350 to 7000 daltons.

19. The formulation of claim 18, wherein the polyethylene glycol ranges in size from about 750 to 5000 daltons.

20. The formulation of claim 3, wherein the polymer is a polysorbate.

21. The formulation of claim 2, wherein the stabilizing agent is a lipid with a lipid to drug weight ratio less than 5:1

22. The formulation of claim 21, wherein the lipid to drug ratio is less than 3:1.

23. The formulation of claim 21, wherein the lipid is selected from natural phospholipids, chemically and enzymatically modified phospholipids, and synthetic phospholipids.

24. The formulation of claim 23, wherein the lipid is a natural phospholipid.

25. The formulation of claim 23, wherein the lipid is a synthetic phospholipid.

26. The formulation of claim 23, wherein the lipid is a diacyl phospholipid.

27. The formulation of claim 26, wherein the lipid is selected from diacyl phosphatidylcholines, diacyl phosphatidylethanolamines, diacyl phosphatidylserines, diacyl phosphatidylinositols, diacyl phosphatidic acids, phosphorylated diacylglycerides, and combinations thereof.

28. The formulation of claim 27, wherein the lipid is a phosphorylated diacylglyceride.

29. The formulation of claim 28, wherein the phosphorylated diacylglyceride is selected from dioleoyl phosphatidylglycerol, palmitoyloleyl phosphatidylglycerol, and combinations thereof.

30. The formulation of claim 27, wherein the lipid is a diacyl phosphatidylcholine.

31. The formulation of claim 30, wherein the diacyl phosphatidylcholine is selected from palmitoyloleoyl phosphatidylcholine, dioleoyl phosphatidylcholine, dilauroyl phosphatidylcholine, dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine, and combinations thereof.

32. The formulation of claim 27, wherein the lipid is a diacyl phosphatidylethanolamine.

33. The formulation of claim 32, wherein the diacyl phosphatidylethanolamine is selected from dipalmitoyl phosphatidylethanolamine, 1-palmitoyl-2-oleoylphosphatidylethanolamine, dioleylphosphatidylethanolamine, and combinations thereof.

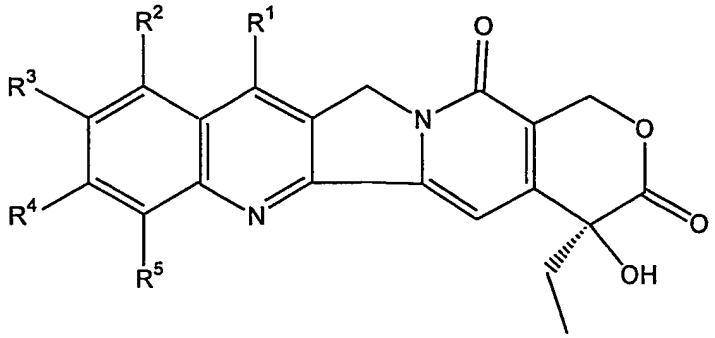
34. The formulation of claim 1, wherein the stabilizing agent is a polymer-lipid conjugate.

35. The method of claim 34 wherein the polymer is polyethylene glycol and the lipid is selected from phospholipids and fatty acids.

36. The formulation of claim 1, wherein the optional excipient is present.

37. The formulation of claim 36, wherein the excipient is selected from polyhydroxyalcohols, saccharides, liquid polyethylene glycols, propylene glycol, glycerol, ethyl alcohol, and combinations thereof.

38. The formulation of claim 1, wherein the camptothecin analog has the structure of formula (I)



(I)

wherein R^1, R^2, R^3, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6} alkylamido, amino, nitro, and cyano, or R^1 and R^2 and/or R^3 and R^4 may together form a substituted or unsubstituted five- or six-membered cyclic group containing up to 2 heteroatoms selected from the group consisting of O, S, and N.

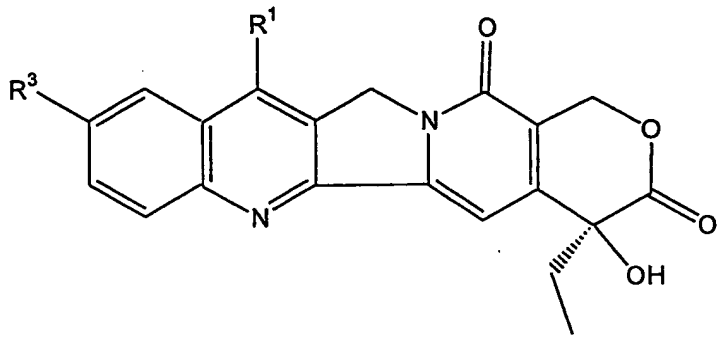
39. The formulation of claim 38, wherein R^1, R^2, R^3, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6} alkylamido, amino, nitro, and cyano.

40. The formulation of claim 39, wherein R^1 is C_{1-6} alkyl, and R^2, R^3, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6} alkylamido, amino, nitro, and cyano.

41. The formulation of claim 40, wherein R^3 is hydroxyl, and R^2, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6} alkylamido, amino, nitro, and cyano.

42. The formulation of claim 39, wherein R^3 is hydroxyl, and R^1, R^2, R^4, and R^5 are independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, acyloxy, hydroxyl, sulfhydryl, acyl, halo, amido, C_{1-6} alkylamido, amino, nitro, and cyano.

43. The formulation of claim 38, wherein R^2, R^4, and R^5 are H, such that the camptothecin analog has the structure of formula (II)



(II)

44. The formulation of claim 43, wherein R^1 is C_{1-6} alkyl and R^3 is hydroxyl, sulfhydryl, or amino.

45. The formulation of claim 44, wherein R^3 is hydroxyl.

46. The formulation of claim 45, wherein the camptothecin analog is 7-ethyl-10-hydroxyl camptothecin.

47. The formulation of claim 1, wherein the formulation is in the form of an aqueous suspension and further comprises an aqueous vehicle.

48. The formulation of claim 47, wherein the aqueous vehicle is water, an isotonic diluent, or a buffer solution.

49. The formulation of claim 1, wherein the formulation is particulate.

50. The formulation of claim 49, wherein the formulation is comprised of particles that have an average size in the range of about 1-1000 nm.

51. The formulation of claim 50, wherein the average size of the particles is in the range of about 50-800 nm.

52. The formulation of claim 47, wherein the aqueous suspension further comprises an acoustically active gas.

53. A method for making a nanoparticulate formulation of a camptothecin analog, comprising:

(a) admixing, in a solvent, a camptothecin analog and a stabilizing agent that stabilizes the camptothecin analog but does not covalently bond thereto;

(b) removing the solvent in a manner effective to provide a dry formulation of the camptothecin analog; and

(c) rehydrating the dry formulation to provide the nanoparticulate formulation.

54. The method of claim 53, wherein the solvent is removed by lyophilization.

55. The method of claim 53, wherein the solvent is removed by spray drying.

56. The method of claim 53, wherein (b) comprises removing the solvent by rotary evaporation, thereby providing an agglomerated intermediate product, and wherein the method further comprises (b') deagglomerating the intermediate product using a procedure effective to provide the nanoparticulate formulation of the camptothecin analog.

57. The method of claim 53, wherein the solute is a supercritical fluid, such as liquid carbon dioxide.

58. The method of claim 53, wherein prior to (a), the step is added of dissolving the camptothecin analog in a first solvent to form a first solution and dissolving the stabilizing agent in a second solvent to form a second solution, and (a) comprises admixing the first solution with second solution.

59. The method of claim 53, wherein an additional component of the stabilizing agent is added during step (c).

60. The method of claim 59, wherein the additional component of the stabilizing agent is a poloxamer and/or a poloxamine.

61. A nanoparticulate formulation of a camptothecin analog prepared according to the method of claim 53.

62. A method for delivering a drug to a mammalian individual to achieve a desired therapeutic effect, comprising administering to the individual a therapeutically effective amount of the formulation of claim 1.

63. The method of claim 62, wherein administration is parenteral.

64. The method of claim 63, wherein administration is intravenous.

65. The method of claim 62, wherein administration is oral.

66. A method for treating an individual suffering from cancer, comprising administering to the individual a spatially stabilized matrix formulation of:

(a) drug-containing particles comprised of (i) a stabilizing agent, (ii) a camptothecin analog that is entrapped by but not covalently bound to the stabilizing agent, optionally (iii) a targeting ligand, and optionally (iv) an excipient selected from the group consisting of saccharides, liquid polyethylene glycols, propylene glycol, glycerol, ethyl alcohol, and combinations thereof, in

(b) an aqueous vehicle suitable for parenteral drug administration.

67. The method of claim 66, wherein the formulation is administered parenterally and the vehicle is suitable for parenteral administration.

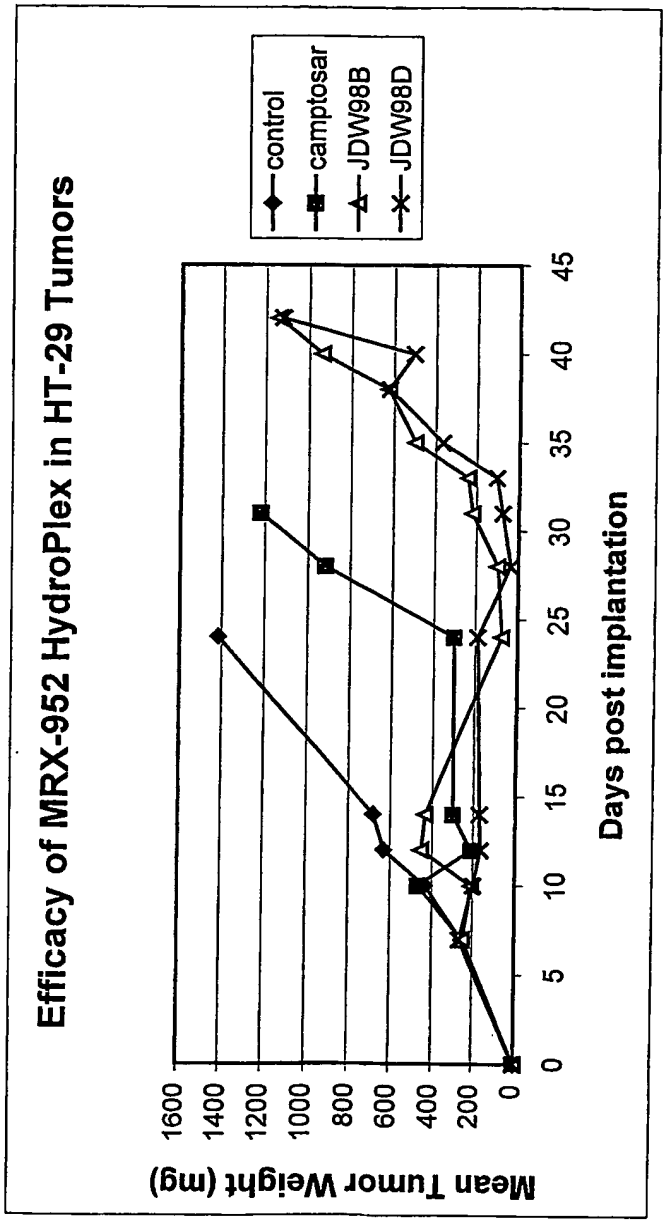

Efficacy of MRX-952 HydroPlex in HT-29 Tumors
control
camptosar
JDW98B
JDW98D
Mean Tumor Weight (mg)
0
200
400
600
800
1000
1200
1400
1600
0
5
10
15
20
25
30
35
40
45
Days post implantation

FIG. 1

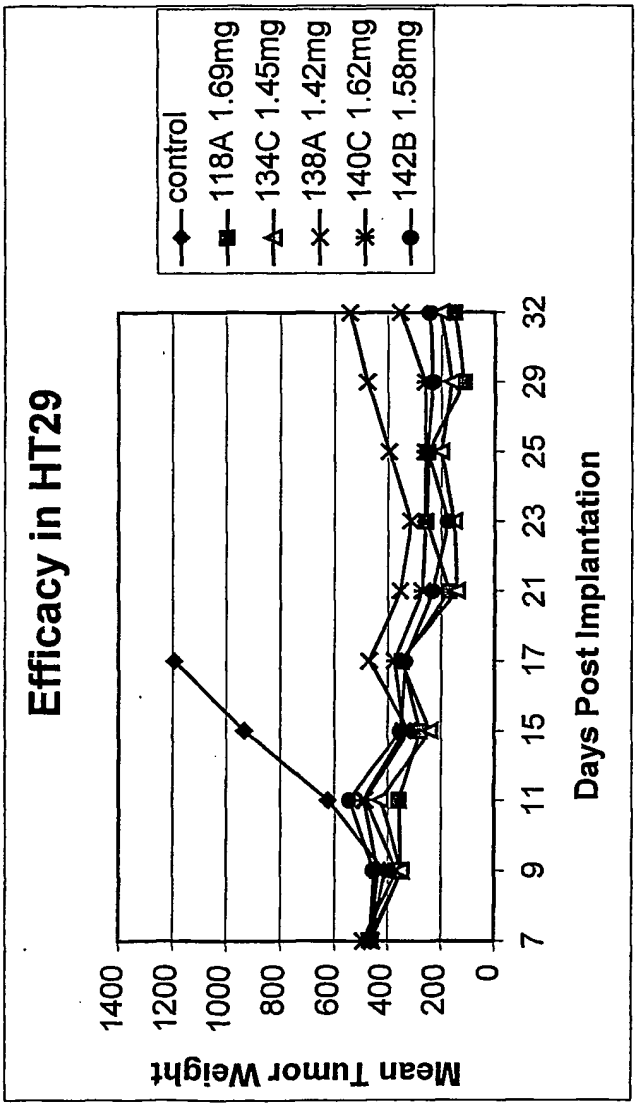


FIG. 2